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Biodegradation of Liquid Gun Propellant Formulation 1846

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LGP is used throughout this document as the notation for Liquid Gun Propellant Formulation 1846.

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Liquid Gun Propellant Formulation 1846 (LGP) is a clear, colorless, odorless liquid. It is a molten salt composed of hydroxylammonium nitrate (HAN, 60.79%, 9.09 molar), triethanolammonium nitrate (TEAN, 19.19%, 1.3 molar), and water (20.02%, 15.93 molar). It is completely miscible with water and the two salts dissociate to yield nitrate and hydroxylammonium and triethanolammonium ions. Although it does not burn unless pressurized, the decomposition progresses to a very energetic reaction when it is ignited at elevated pressure in a confined space (as in the breech of a gun). The properties of LGP, including safety, cost, and performance, have led to its selection by the Army as the propellant for a new 155 mm howitzer. In support of the ongoing development program for this new weapon system, the U.S. Army Environmental Center (USAEC) conducted an evaluation of the biodegradability of LGP. This research and development effort was conducted in support of the Armament Research, Development and Engineering Center (ARDEC) which is completing a life cycle assessment for the LGP Program. The laboratory studies reported in this document were completed by IT Corporation for the U.S. Army Environmental Center (USAEC) under Contract No. DACA31-91-0047, Task Order No. 2.

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The large-scale use of LGP will inevitably result in the generation of LGP-contaminated materials that will require treatment or disposal. These materials could include: 1) contaminated LGP, 2) LGP that is otherwise out of specification (e.g., beyond shelf life) or 3) water, soil or debris contaminated with LGP. For example, wash water contaminated with LGP is generated when the gun and its components are cleaned during test firing of the system. Soil or water could be contaminated if LGP were spilled during production, shipment, storage or field use. Information on effective means of treating this material, if required, is needed. Previous research indicated that biodegradation is a technology that warrants further study and that bench-scale studies should be conducted to prove the feasibility of microbial degradation. The objective of the laboratory evaluation documented in this report was to assess the potential effectiveness and applicability of biological treatment for LGP.

Specific activities conducted during this project were:

- Screening and selection of microbes capable of tolerating LGP;
- Development of an analytical method capable of quantifying low levels of LGP in environmental samples;
- Evaluation of degradation of LGP in soil and water matrices;
- Evaluation of the effectiveness of Sequencing Batch Reactor (SBR) for treatment of LGP in an aqueous matrix.

Upon initiation of the laboratory studies, it was found that adequate analytical methods did not exist for quantifying low concentrations of HAN and TEAN present in environmental samples. It was subsequently learned that the Army Corps of Engineers Waterways Experiment Station (USAWES) was also pursuing lower detection limits for LGP components and had initiated development of a High Performance Liquid Chromatography (HPLC) method in this regard. Information was shared and an analytical method that met project needs was developed. In addition to the assessment of biodegradability of LGP, the advancement of development and validation of this analytical method is a significant contribution of this research and development effort.

Based on the results of the laboratory investigation conducted, it was concluded that:

- LGP is toxic or inhibitory to soil microbes at levels above 800 ppm; however, a toxicity threshold was not determined.
- The HPLC analytical method initially developed by USAWES and further refined under this effort provides the capability to quantify low levels of HAN and TEAN in environmental samples.
- HAN is quickly degraded in environmental samples by physical and chemical reactions.
- TEAN is recalcitrant to biodegradation.
- When added to soil and water matrices, LGP acidifies the sample.

Recommendations for further study include:

- Further investigation of the feasibility of biodegradation of TEAN using SBRs and a longer Biological Solids Retention Time (BSRT) (e.g., 40 days).
- Completion of a full validation of the USAWES HPLC analytical method, including interlaboratory studies with round-robin analysis. The identification and resolution of matrix interferences should be included.
- Development of a standardized method for extraction of LGP from soils.

The research efforts conducted during this project were completed by IT with direction from USAEC, Environmental Technology Division, Technology Demonstration and Transfer Branch. Samples of HAN, TEAN, and LGP and methods for appropriate handling were supplied by the Army's Ballistics Research Laboratory (BRL). The U.S. Army Construction Engineering Research Laboratory (USACERL) served in a technical advisory role throughout the study. USAWES provided technical information regarding related development of analytical methods. Information regarding the LGP Program was supplied by ARDEC during periodic project briefings.

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List of Abbreviations

BOD_5	5-day biological oxygen demand
BSRT	biological solids retention time
COD	chemical oxygen demand
CFU/mL	colony-forming units per milliliter
DEA	diethanolamine
DI	deionized water
EA	ethanolamine
F:M	food to microorganism ratio
g/cc	grams per cubic centimeter
HAN	hydroxylammonium nitrate
HRT	hydraulic retention time
kg	kilogram
L	liter
LGP	liquid gun propellant formulation 1846
mg/L	milligram per liter
mg/kg	milligram per kilogram
mL	milliliter
MLVSS	mixed liquor volatile suspended solids
mL/min	milliliter per minute
NOD	nitrogenous oxygen demand
POTW	publically owned treatment works
ppb	parts per billion
q	specific substrate utilization rate
rpm	revolutions per minute
SBR	sequencing batch reactor
TEAN	triethanolammonium nitrate
TKN	total Kjeldahl nitrogen
TIC	total inorganic carbon
TOC	total organic carbon
TSS	total suspended solids
WAS	waste activated sludge
μg/L	micrograms per liter
USACERL	U.S. Army Construction Engineering Research Laboratory
USAEC	U.S. Army Environmental Center
USAWES	U.S. Army Waterways Experiment Station

1.0 Introduction

Liquid Gun Propellant Formulation 1846 (LGP) is a clear, colorless, odorless liquid. Although it does not burn unless pressurized, its decomposition progresses to a very energetic reaction when ignited under pressure in a confined space (as in the breech of a gun). Properties of LGP, including safety, cost, and performance, have led to its selection by the Army as the propellant for a new 155 mm howitzer. Previous research conducted by the U.S. Army Environmental Center (USAEC) concluded that biodegradation offers the greatest potential for treatment of LGP residues.[1] As a result, the USAEC conducted a laboratory investigation to evaluate biodegradation of LGP. This research and development effort was conducted in support of the Armament Research, Development and Engineering Center (ARDEC) which is completing a life cycle assessment for the LGP Program. The laboratory studies reported in this document were completed by IT Corporation for the U.S. Army Environmental Center (USAEC) under Contract No. DACA31-91-0047, Task Order No. 2.

LGP is a molten salt composed of hydroxylammonium nitrate (HAN, 60.79%, 9.09 molar), triethanolammonium nitrate (TEAN, 19.19%, 1.3 molar), and water (20.02 %, 15.93 molar).[2] It is completely miscible with water and the two salts dissociate to yield nitrate and hydroxylammonium and triethanolammonium ions. The composition of LGP and the chemical structure of HAN and TEAN are shown in Figure 1. The active ingredients, being salts, have no appreciable vapor pressure. LGP has a density of 1.430 g/cc at 25°C. No freezing point has been observed for LGP; its boiling point has been calculated to be 123.7°C at standard pressure (760 mm).[2] Although the propellant can fume if heated, it does not burn unless pressurized and does not have a measurable flash point. Oxidizing and reducing agents will react with HAN-based propellants.

The large scale use of LGP will inevitably result in the generation of LGP contaminated materials and residues that will require treatment or disposal. These materials could include: 1) contaminated LGP, 2) LGP that is otherwise out of specification (e.g., beyond shelf life) or 3) water, soil or debris contaminated with LGP. For example, wash water will be contaminated with LGP when an LGP fired howitzer and its components are cleaned after firing. Additionally, soil or water could be contaminated if LGP were spilled during production, shipment, storage or field use. Information on effective means of treating this

material is needed. Previous research indicated that biodegradation is a technology that warrants further study and that bench scale studies should be conducted to prove the feasibility of microbial degradation.[1] The objective of the laboratory evaluation documented in this report was to assess the potential effectiveness and applicability of biological treatment for LGP.

Kaplan examined the biodegradation of pure derivatized ammonium nitrate propellants and HAN.[3] The following conclusions were drawn from this previous study of the persistence and fate of HAN and various derivatized ammonium nitrates in soil and bioreactors:

- HAN was unstable at a pH greater than 5.9 and decomposed to yield nitrate and hydroxylamine. It was concluded to be nonpersistent under most environmental conditions.
- Biodegradation of pure TEAN was demonstrated at concentrations of 50, 500, and 5000 mg/kg in soil.
- Mineralization of the organic moiety of TEAN was demonstrated using ^{13}C-labeled TEAN.
- Biodegradation of TEAN was evaluated at various hydraulic retention times in continuous flow bioreactors. However, several critical operating parameters were not reported (e.g., biological solids retention time (BSRT), food to mass ratio, and solids analysis).
- A strain of *Hyphomicrobium* was isolated which could biodegrade several derivatized ammonium nitrates, including TEAN.

When the current study was initiated, the information summarized above represented the state of knowledge regarding the biodegradation and environmental persistence of the constituents of LGP. However, a limitation of the previous studies was that the biodegradation of HAN and TEAN had only been evaluated separately and not as combined in LGP.[2] Whether a synergistic positive or negative effect on reaction rates or microbial toxicity might occur had not been determined.

Early in the current investigation, it was found that adequate analytical methods existed for quantifying high concentrations (>100 mg/L) of HAN and TEAN in clean samples (e.g., LGP or deionized water). However, as discussed later in this report, the capability to quantify significantly lower concentrations of these constituents in environmental matrices

became a necessity. Analytical methods that could meet this requirement did not exist. In particular, methods that would allow quantification of TEAN in the 1 to 10 mg/L range were not available. Therefore, the USAEC modified the scope of the original Task Order to include development of needed analytical capabilities. Subsequent communications with the U.S. Army Waterways Experiment Station (USAWES) revealed that USAWES was also pursuing methodology that could achieve lower detection limits for LGP components.[4] USAWES had initiated development of a High Performance Liquid Chromatography (HPLC) method in this regard. Information was shared and an analytical method that met project needs was developed. In addition to the assessment of biodegradability of LGP, advancing the development and validation of this analytical method became a significant contribution of this research and development effort. Analytical methodology is discussed in Section 3 and Appendix A.

Degradation of LGP in soil, groundwater, and in an aqueous solution treated in a bioreactor is discussed in Sections 4, 5, and 6, respectively. The results presented in these sections indicate important concerns related to the biodegradability of LGP and the overall potential for using biodegradation to treat LGP-contaminated soil, water, and other contaminated media. Conclusions and recommendations for future research are presented in Section 7.

In addition to the summary of information presented in this report, supplemental information related to the laboratory investigation is contained in separate, unpublished documents submitted during the course of the study. Significant project documents include:

- Test Plan for LGP Biodegradation Studies, May 1992
- Accident Prevention Safety Program Plan for LGP Biodegradation Studies, April 1992
- Documentation of Existing Methods for Quantitation of TEAN, November 1992
- Assessment of Validity of Ion Chromatographic Method for Determining HAN and TEAN in Soil, Water, and Nutrient Broth, November 1993 (Appendix A)

The research efforts conducted during this project were completed by IT under the direction of the USAEC's, Environmental Technology Division (ETD), Technology Demonstration and Transfer Branch. Samples of HAN, TEAN, and LGP and methods for handling and storage were supplied by the Army's Ballistics Research Laboratory (BRL). The U. S. Army Construction Engineering Research Laboratory (USACERL) served in a technical advisory

role throughout the study. The Army Corps of Engineers Waterways Experimental Station (USAWES) provided technical information regarding ongoing development of analytical methods. Information regarding the LGP Program was supplied by ARDEC.

2.0 Selection and Screening for LGP Tolerant Microbes

Isolation of bacteria capable of tolerating and biodegrading LGP was the first objective of the LGP biodegradation study. Previous research conducted by Kaplan included the isolation of a strain of *Hyphomicrobium* which was reported to be capable of degrading several derivatized ammonium nitrates (including TEAN).[3] However, communications with Dr. Kaplan revealed that microbial cultures used in the earlier studies had not been maintained and it became necessary to obtain microbes from other sources.[5]

Discussions were held with USAEC, ARDEC, and BRL personnel to determine if LGP contaminated soil existed at any test area. Such samples might have yielded LGP tolerant microbes that had evolved through natural selection processes. However, such materials were not available. Therefore, a strategy for acquisition and selection of microbes from other sources was implemented. In order to increase the chances of finding bacteria that could grow in the presence of LGP and hopefully biodegrade it, multiple soil, sludge and compost samples were used as initial sources of microbes. Both clean and environmentally impacted samples were collected from 14 sites (Table 1). The selection and screening strategy involved testing isolates from each soil for tolerance to LGP at various concentrations. Both aerobic and anaerobic cultures were sought.

A flow chart describing the bacteria selection procedure is shown in Figure 2. The first attempt to select LGP-degrading bacteria was performed by preparing a mineral salts medium (Appendix B) supplemented with 1,000 mg/L LGP. Based on information published in the literature, it was anticipated that this concentration of LGP would be selective but not toxic.[3] In addition to the LGP medium, additional media were prepared that were also supplemented with either a simple carbohydrate, alcohol, or other carbon source (i.e., glucose, methanol, or acetate, respectively). This approach was adopted in the event that LGP did not provide a suitable carbon and energy source for microbial growth. Each medium was inoculated with 5 grams of soil from the sources identified in Table 1. Each soil source was separately inoculated into duplicate tubes of medium. One half of the tubes were incubated aerobically and the other half were incubated in an anaerobic glove box to encourage denitrification. Incubation was conducted at room temperature. After 15 days there was no indication of growth.

LGP is acidic and the addition of 1,000 mg/L LGP to the culture medium resulted in an acidic medium (~ pH 5). To eliminate the possibility that this low pH had inhibited growth, a second experiment was conducted using the same medium adjusted to pH 7. Culture conditions were identical to the first experiment, and again no increase in turbidity (i.e., no significant microbial growth) was observed. However, microscopic examination indicated that viable bacteria were present. Subsamples were collected from each tube and spread onto solid LGP medium (mineral salts, 1.5% agar, 1000 mg/L LGP) and nutrient agar medium (Appendix B). In all cases, the bacteria failed to grow on the LGP medium (1,000 mg/L LGP) but grew on the nutrient agar medium. Thus, viable bacteria could be recovered from the LGP media but bacterial growth was not observed in LGP solids. This observation suggested that the bacteria could survive at a concentration of 1,000 mg/L but that LGP inhibits growth at this concentration.

Individual colonies were isolated from each nutrient agar plate representing at least one isolate from each soil sample. These isolates were then further selected for tolerance to LGP. Each of these isolates was initially cultured in a medium composed of 2,000 mg/L nutrient broth and 100 mg/L LGP. Subsequently, isolates were selected for their tolerance to LGP by incrementally increasing the LGP concentration and decreasing the nutrient broth concentration. As indicated in Figure 3, six strains were isolated that tolerated LGP at concentrations greater than 300 mg/L and three strains would grow at concentrations as high as 800 mg/L. It was found that the lowest concentration of nutrient broth that would sustain microbial growth in the presence of LGP was 1,200 mg/L. These strains were maintained in 1,200 mg/L nutrient broth and their highest tolerated concentration of LGP. None of the strains grew in the presence of LGP when glucose, methanol, or acetate was substituted for nutrient broth. Each of the six strains was aerobic; anaerobic microbes capable of surviving under these conditions were not found.

The results observed during the bacteria isolation and selection process indicated that significantly lower concentrations of LGP would be used in the biodegradation studies than originally planned. As discussed in Section 3, this finding necessitated development of an analytical methodology capable of quantitating low concentrations of HAN and TEAN. During this period (several months), selective pressure was exerted on the isolated strains by a program of gradually decreasing the concentration of nutrient broth while increasing the concentration of LGP.

The analytical method described in Section 3.0 was utilized to assess the ability of selected bacteria to biodegrade LGP. The concentration of HAN and TEAN was measured over a period of 33 days while bacterial cultures were growing in 2,000 mg/L nutrient broth containing the maximum concentration of LGP in which the cultures could survive. Two cultures, No. 1 and No. 2, were grown in medium initially containing 800 mg/L LGP, Culture No. 3 and Culture No. 5 were grown at 700 mg/L and 600 mg/L respectively, and Culture Nos. 4 and 6 were grown in 400 mg/L LGP. The results, shown in Table 2, indicate that HAN was completely degraded during this microbe selection period. The analytical method for HAN quantifies the dissociated ions (nitrate and hydroxylammonium). Since only nitrate was present, it was concluded that the disappearance of HAN was due to degradation and not merely dissociation. TEAN was recalcitrant and no degradation was observed after 33 days.

Based on the results of the screening process described above, three aerobic microbial cultures were selected for use in the biodegradation testing due to their tolerance to LGP. These strains, derived from a crude oil degrading strain, mushroom compost, and Bunker-C impacted soil, were designated Culture Nos. 1, 2, and 3, respectively. Cultures 1 and 2 were determined to be pseudomonas; the identity of the third culture could not be determined (Table 3). Characterization of the microbes was conducted using gram staining techniques, microphotography, and API® microbial identification test kits (Analytab Products Division of Sherwood Medical). Membrane lipid analyses (Microcheck®) were also conducted; however, the results obtained from these tests were spurious and not consistent with organisms that would be found in soil environments (e.g., one culture was identified as the pertussis, whooping cough, bacterium). Therefore, these were not deemed to be valid.

3.0 Analytical Methods Development and Testing

3.1 Method Development

A literature review identified several potential methods for the quantification of HAN and TEAN. The methods evaluated included thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), infrared spectroscopy (IR), and potentiometric titration. Optimum methodology for HAN was determined to be oxidation of HAN to nitrous oxide and detection by GC (ppb quantification).

The analytical methods for TEAN published by D. L. Kaplan,[3] although successfully reproduced in the laboratory, did not provide the necessary detection levels (10 mg/L). Additionally, the method was subject to considerable matrix interferences from the supplemental carbon source (nutrient broth) and was suitable only for analysis of clean, concentrated solutions of LGP. Evaluation of several other methods for TEAN analysis also provided unsatisfactory quantification of TEAN.

Communication with researchers at USAWES, who were performing unpublished research involving low concentrations of LGP in deionized water, indicated that with an electrochemical detector, detection limits of 20 and 220 μg/L could be achieved for HAN and TEAN, respectively.[4] Furthermore, problems with interferences had not been encountered. Review of the method description and the equipment specifications indicated the USAWES method offered the most potential for detection of TEAN at low concentrations.

Methods development and testing activities associated with this project are documented in a separate project report (Appendix A). The available analytical methods, their performance, the testing of new HPLC based ion chromatography procedures, and the validation of the HPLC based method developed by USAWES for the detection of low concentrations of HAN and TEAN are described in Appendix A. The results of the method development, testing activities, and evaluation are summarized in this section. Detailed procedures and performance data are included in Appendix A. (For convenience, this method is referred to as the USAWES method in this report. The method has not been formally validated or assigned a specific name or identifier.)

The USAWES method was developed for detection and quantification of low concentrations of TEAN and HAN in environmental samples. The method, based on ion chromatography, employs a cation exchange column that simultaneously separates mono- and divalent cations and low molecular weight amines and alkanolamines. Following elution from the column, the sample is mixed with sodium hydroxide via a post-column reaction system before passing through the detector. A pulsed electrochemical detector, with a gold working electrode and a sodium hydroxide saturated sodium chloride/silver chloride reference electrode, was used to detect HAN and TEAN.

Detector linearity was documented over a range of 3 to 30 mg/L for HAN and 1 to 10 mg/L for TEAN. A minimum detection limit for HAN was calculated to be 20 μg/L using three times the background noise level. The minimum detection limit for TEAN was calculated to be 220 μg/L. The retention time for HAN was 3.33 minutes and 6.42 minutes for TEAN. Variability of triplicate analysis of the same sample was reported to be less than 3 percent for HAN and less than 7 percent for TEAN.[4] These results were obtained using TEAN and HAN solutions prepared in deionized water.

The USAWES method proved to be useful for the detection of LGP, ethanolamine, and diethanolamine. A detection limit of 1 mg/L for both HAN and TEAN was achieved in aqueous samples; however, a quantifiable limit of 1 mg/L for TEAN was difficult to achieve on a routine basis. The quantifiable limit for TEAN typically ranged between 1 and 3 mg/L. Based on the laboratory experience acquired during this study, it should be noted that the USAWES method was complicated to reproduce and requires sophisticated detection equipment, a specific cation exchange column, a well controlled post-column chemical reaction to ionize target compounds, and skilled analysts to execute the method.

The method also requires a relatively high level of maintenance to insure satisfactory performance. Empirical evidence indicates that a new cation exchange column will support the analysis of 300 to 400 samples before performance degrades. Attempts to restore used columns were not successful. The detection electrode requires maintenance every few weeks depending on the number of samples analyzed. Specifically, the electrode must be rebuilt to replenish electrolyte solution, polish the gold working electrode, and replace the semi-permeable membrane separating the reference electrode, sample chamber, and working electrode. The highly active surface of the column can attract contaminants from

environmental water and soil samples. These accumulate on the column resulting in lowered resolution and shortened column life. The short life of the cation exchange column and the complex maintenance requirements of the electrode make the method challenging to reproduce and perform on a routine basis.

The USAWES method was tested by evaluating its ability to quantitate HAN and TEAN in a variety of aqueous and soil matrices. Matrix interferences were encountered, for example, due to large concentrations of sodium in seawater samples, but were not observed with the lower concentrations of ions present in nutrient broth. Reproducibility was poor at lower concentrations of HAN and TEAN for every environmental matrix.

With proper attention to Quality Assurance and column and detector maintenance, the method provided acceptable precision to determine the biodegradability of HAN and TEAN since relative changes can be used to quantitate HAN and TEAN biodegradation. The quantifiable range was 1 to 400 mg/L for TEAN, 1 to 150 mg/L for HAN, 0.25 to 25 mg/L for EA, and 0.5 to 75 mg/L for DEA. For further details on analytical methods and method performance, refer to Appendix A. The detector response was linear over the entire quantifiable range. Care was exercised to select test matrices that resulted in the least amount of interference. This approach helped reduce the confounding effects of sample interference and chemical instability on the evaluation of biodegradation.

Nitrate and nitrite were quantitated using a Dionex AS4C-SC 4-mm microcolumn with a guard column, a conductivity detector, and an anion micromembrane suppressor with a 4 mL/min flow of 50 mM sulfuric acid. The eluent was 1.8 mM sodium carbonate, 1.7 mM sodium bicarbonate solution flowing at 1.5 mL/min.

3.2 Quality Control

Five-point calibration curves were used for each compound. HAN and TEAN calibration data were generated using standards containing 200, 100, 40, 10, and 2.5 mg/L of each component. A calibration curve was generated for each run. Nitrate and nitrite calibration data were generated using standards containing 80, 40, 10, 1, and 0.5 mg/L.

Matrix spikes and standard addition samples were prepared during each sampling event for HAN, TEAN, DEA, EA, nitrate, and nitrite. Matrix spikes for HAN and TEAN consisted of adding 400 mg/kg of each to a randomly selected duplicate soil or water sample. The

matrix spike was extracted and analyzed in the same manner as the other samples. Standard additions of 80 mg/L HAN and TEAN were also added to 5 mL of soil extract or water, as appropriate. Method blanks were lab water or extracts of untreated soil. Instrument performance and quality of the calibration curve were validated by analyzing a single calibration standard at a 10 percent frequency and at the end of each set of samples. The same protocol was used for nitrate and nitrite analyses. Matrix spikes and standard additions contained 360 mg/kg of HAN and TEAN and 40 mg/L of HAN and TEAN, respectively. All check standards were within ± 10 percent.

4.0 Biodegradation of LGP in Soil

An investigation of the ability of LGP tolerant microbes to degrade LGP in a soil matrix was conducted (water matrix tests are discussed separately in Section 5). Research into bioremediation involving soils is inherently difficult due to the complex ion chemistry, large surface area, and hydrations which can shield compounds from extraction. Initial screening studies were conducted using soils that could be categorized as sand, clay, and organic type soils. Samples of a sandy soil were obtained from a shallow aquifer near Chicago, Illinois. Clay soil was collected from a site in Blount County, Tennessee, and common commercially available potting soil was used as the organic soil. These soil types presented a broad range of characteristics.

4.1 Soil Screening and Selection

Literature reviews and communication with USAWES scientists indicated that procedures for extraction of LGP from soils have not been defined or tested.[4] Similarly, the potential for interferences in a complex extract had not been carefully examined. The extraction efficiency of LGP components from different soil types had not been previously assessed. Therefore, the preparation and analysis of soil extracts represented a significant component of the soil screening and selection which was conducted as part of the USAWES method validation effort (additional details are contained in Appendix A).

Three solvents were initially examined for their extraction capability: deionized (DI) water, potassium chloride, and methanol. DI water was evaluated because of its effectiveness in stripping ions from soils by establishing a severe concentration gradient between soil particles and the aqueous phase. A solution of 3M potassium chloride is a common soil extractant used for cations. This solution can be effective in displacing adsorbed cations with potassium. Since HAN and TEAN are soluble in alcohols, methanol was also considered to be a potential extracting solvent. Analytical interferences caused by these solvents were not observed. As documented in Appendix A, deionized water provided the best recovery of the extraction solvents tested.

The quantifiable limit for LGP varied with soil type because the dilution required to achieve a workable solvent volume varied with the soil type. For example, the quantifiable limit for TEAN in clay was higher than for TEAN in sand because it was necessary to add more

water to the clay soil to produce an extraction mixture that had recoverable water. Eight mL of water was required for each gram of clay soil, whereas only 2.5 mL was required to give excess water in sand and organic soil samples.

The observed inability to recover low concentrations of HAN and TEAN from clay and organic soil suggested that these compounds were either not stable or resisted extraction due to chemical interactions with soil particles. The results of extracting HAN and TEAN with DI water from soil immediately after spiking (Table 18 of Appendix A) provided improved recovery when compared to extraction of samples that had been allowed to age for seven days.

To further investigate the effectiveness and efficiency of using DI water to extract HAN and TEAN, aliquots of the sand, clay, and organic soils were spiked with LGP and analyzed in triplicate. Prior to spiking, the soils were dried, sieved and homogenized. The soils were then rehydrated with LGP spiked water and thoroughly mixed to a homogeneous consistency. Each soil type was spiked with LGP to 2, 10, and 100 times the quantifiable limit for the soil, accounting for dilution. Two sets of triplicate samples of spiked soil were prepared. One set was stored at 4°C for six days prior to extraction with aqueous solution and the other was extracted immediately. The results (shown in Tables 19 and 20 of Appendix A) suggest that HAN and TEAN are not stable in or not extractable from clay and organic soil. Recovery of high concentration LGP spikes suggested that oxidatively reactive compounds in the soil were expended by less than the maximum amount of LGP added to the soil.

Extraction efficiency of DI water was determined using triplicate spiked samples. The LGP was allowed to contact the soil matrix for six days at 4°C to facilitate adsorption. Each soil matrix was then extracted and the amount of HAN and TEAN recovered was compared to the amount added. Variability among triplicate samples was also determined. The results indicated low recovery of low concentrations of both TEAN and HAN in each soil type. HAN recovery from the sand and organic soil was low at each concentration tested. Recovery from the clay soil increased with concentration. TEAN recovery exceeded 100 percent in several cases, suggesting interference with TEAN analysis was caused by soil specific interactions. Therefore, the clay soil was selected as the test matrix for LGP biodegradation studies because of superior HAN and TEAN recovery, persistence, and reproducibility.

4.2 Experimental Design for Soil Biodegradation Study

The intent of the soil biodegradation study was to evaluate persistence of the LGP in soil. During preparation of the soil aliquots, the soil was first dried and then screened through a 6.3 mm mesh to remove rocks and pebbles and to homogenize the sample. Fourteen soil treatments were prepared: treatments LT1 through LT8 were prepared using nonsterile soil (i.e., soil containing active microbial populations), treatments LT9 through LT14 included autoclaving the soil for 2 hours to reduce the natural bacterial population (Table 4). (Complete sterilization of soil is difficult due to heat transfer properties of soil; however, autoclaving reduced the bacterial population density in the soil from approximately 1 million colony forming units (CFU) per gram to below detection limits.) A total of 2.3 to 2.5 kg of soil was distributed into each of 14 containers. Each container was amended with 500 mg/kg RESTORE®, 50 g powdered lime, and 800 mg/kg LGP. (RESTORE® is a trademark product of IT Corporation, it consists of a mixture of soluble ammonium and phosphate and is used in insitu aquifer treatment systems.) Selected treatments (as shown in Table 4) were also amended with bacterial cultures No. 1, 2, and 3 from the screening study to a concentration of $5x10^7$ CFU/kg.

The soil was manually mixed five days per week. Once each week, an aliquot of 100 g of soil was removed from each container and extracted with deionized water. HAN, TEAN, nitrate, nitrite, ammonium, pH, and soil moisture were measured in the extract. Microbial population density and phosphate content were determined on alternating weeks. The analytical methods used for each test are listed in Table 5.

The soil biodegradation test was continued for 7 weeks at which time neither HAN and TEAN were detected in the soil. Throughout the test, soil moisture was maintained at 15 to 20 percent by weight and temperature was maintained at 23°C. Additional nutrient amendments and pH adjustments were not made.

4.3 Results of Soil Biodegradation Tests

4.3.1 LGP Biodegradation

Weekly analysis of the HAN and TEAN concentrations in the soil was the primary criteria used to evaluate the degradation of LGP. Nitrate and nitrite were also measured. Nitrate ions should be released if HAN and TEAN dissociate or biodegrade. Nitrite is an

intermediate formed from nitrate during denitrification. Formation of significant concentrations of nitrite is a potential, undesirable consequence of LGP biodegradation.

Based on the composition of LGP, spiking the soil with 800 mg/kg LGP would have contributed 152 mg of TEAN and 488 mg of HAN to each kg of soil. However, concentrations less than these expected values were recovered. HAN and TEAN were observed to have varying degrees of chemical stability in the different soils. The initial loss of HAN and TEAN is attributed to physical or chemical decomposition and, to a lesser degree, adsorption. This phenomenon was also observed during analytical method testing (Appendix A).

HAN was observed to quickly dissipate in soil (Figure 4). The inability to recover HAN during the biodegradation experiments could be due to chemical decomposition or to irreversible adsorption to soil particles, glass containers, bioreactors, or biomass. HAN is known to be very unstable.[3,6] Evidence that HAN was degrading rather than being adsorbed can be derived from the method validation work (Appendix A, Tables 19 and 20). Results indicate that recovery of higher concentrations of HAN (256 mg/kg or greater) from clay soil was possible using water as the extractant even after 6 days of equilibration (72 percent or better). These observations, the documented instability of HAN, and no observations of adsorption saturation suggest that adsorption was not the principal mechanism of HAN disappearance in this investigation. It was concluded that HAN degraded or chemically decomposed. The lack of observable differences in the degradation of HAN among the treatments, the instability of HAN observed during analytical method testing, and the published literature on the persistence of HAN suggests that the observed loss of HAN was primarily due to undefined physical or chemical processes rather than biodegradation. Published work also indicates that this process is further enhanced if the soil has a neutral or alkaline pH.[3,6] The pH of the soil had been neutralized with lime to encourage microbial activity.

The initial concentration of TEAN in soil extracts was lower than expected (based on the amount added); however, after the initial loss residual TEAN persisted with little or no reduction in concentration for three to five weeks (Figure 5). The initial loss is likely due to the efficiency of the extraction procedure and/or some loss due to adsorption of TEAN on soil particles. However, after three weeks, a decrease in TEAN concentration was

observed in the native soil treatments (Figure 5). A similar response was observed after 4 to 5 weeks (Figure 5) in autoclaved soil samples which had been amended with LGP tolerant cultures.

The observed TEAN degradation appears to be the result of biological activity and not due to adsorption. The abrupt change in TEAN concentration after 4 to 5 weeks indicates that the soil microbiota had become competent for TEAN biodegradation. The time lag prior to TEAN degradation indicates that an acclimation period was required for soil microbes to degrade TEAN. This loss after a lag time is not consistent with adsorption, which would cause an initial loss to occur until equilibrium is reached.

As seen in Figure 6, the size of the bacterial population increased during the test. The increase can be directly correlated with the loss of TEAN. This observation further supports biodegradation as the removal mechanism and suggests that the presence of a relatively large, growing bacterial population is necessary for TEAN biodegradation.

The autoclaved soil had a very low initial bacterial population. The added strains appeared to have poor survivability in the soil. This is especially evident in the autoclaved soil where the bacterial density was well below expected concentration based on the number of bacteria added. In time, the bacterial population in these samples rebounded to levels identical to the native soil, and this increase appeared to be associated with TEAN degradation. Based on these observations, the LGP tolerant strains added to the autoclaved soils did not enhance LGP biodegradation.

4.3.2 Nitrate and Nitrite Concentrations in Soil

When TEAN and HAN dissociate, nitrate is released. Nitrate can be utilized by microbes; however, it is not the preferred nitrogen source for aerobic growth. Nitrate based respiration occurs under anaerobic conditions, therefore, the aerobic conditions used during the soil treatment tests were not conducive for nitrate utilization. Changes in nitrate were measured during the soil biodegradation test. Based on the composition of LGP and the amount added to the soil, 335 mg/kg of nitrate were added to the soil samples. The measured concentration of nitrate was approximately 300 mg/kg and was constant during the study. This concentration is consistent with the expected concentration. Observed nitrite concentrations suggested that a small amount of the nitrate was being reduced to

nitrite (Figure 7). Nitrite was apparently consumed since the concentration reached an equilibrium at 2 to 3 mg/kg. High levels of nitrite did not accumulate in the experimental treatments, each of which behaved similarly in this regard. There were no obvious trends in the nitrate and nitrite data that distinguished one treatment from the others.

4.3.3 Nutrient Utilization and Soil pH

Ammoniacal nitrogen (AN) was utilized much more rapidly in the native soil treatments than the autoclaved soil (Figure 8). The observed change in AN concentration correlated with the increase in microbial population density. The native soil used AN at a faster rate than the autoclaved treatments. This is consistent with the bacterial population growth observed for the autoclaved treatments (Figure 6). A minimum concentration of 15 to 20 mg/kg AN was reached in most treatments. The lack of further utilization of AN suggests that the AN concentrations reached a lower threshold below which soil microbes could not or did not use the residual ammonium. This may simply be a function of the soil type since sorption characteristics vary by soil type.

The phosphate concentration in each treatment generally declined during treatment (Figure 9). No association between phosphate concentration, microbial population density, or LGP biodegradation was observed other than the general utilization of phosphate and bacterial growth.

Addition of LGP to the soil resulted in an acidic pH. Addition of lime neutralized the soil pH which ranged between 6.5 and 7.5 for the duration of the test (Figure 10).

4.3.4 Toxicity of Remediated LGP Contaminated Soil

Analytical results indicated that concentrations of both HAN and TEAN fell to below detection limits in all treatments. Because residual LGP or undefined by-products could persist in the soil, a cursory assessment of toxicity of the soil toward plants was tested using a very simple seed germination test. Kentucky 31 Fescue seeds were planted in soil from each treatment. Figure 11 shows the germination rate in each treatment. Germination ranged from 70 to 90 percent, which is considered acceptable for viable commercial seed stock. None of the treatments showed significant toxicity toward the fescue embryos or seedlings. The grass continued to grow after germination. Visual examination revealed no differences in the young plants.

4.4 Summary

The results from the soil treatment tests indicate that low concentrations (<800 mg/kg) of LGP can be treated in soil. The results from the soil biodegradation test are summarized below:

- HAN disappears from soil rapidly. Although the mechanism was not defined, it is believed to be physical decomposition (e.g., hydrolysis) and not adsorption.
- TEAN is not subject to complete physical decomposition although some decomposition or adsorption appears to occur upon contact with soil.
- TEAN persisted in the soil for three to five weeks before being degraded.
- Microbial growth coincided with TEAN degradation, suggesting that TEAN was biodegraded.
- LGP tolerant strains did not enhance biodegradation of HAN and TEAN.
- Adjustment of the soil pH was required due to the acidification of the soil caused by addition of LGP.
- Preliminary assessment of the treated soil did not indicate a toxicity toward germination of grass seed and plant growth.
- Nitrate concentrations were constant during treatment. Nitrite accumulated to concentrations of 2 to 3 mg/kg.

5.0 Biodegradation of LGP in Aqueous Samples

In addition biodegradation of LGP in soil, biodegradation of LGP in aqueous samples is also of interest. During the storage, transport, and use of LGP, the potential exists for spills resulting in contamination of inland surface water, ground water, or seawater. Knowledge regarding the fate of LGP in aqueous matrices is therefore important to the development of the LGP program.

5.1 Preliminary Assessment of LGP Stability in Aqueous Matrices

The initial evaluation focused on two types of water: ground water and seawater. Samples of groundwater were obtained from a potable water well located in Knox County, TN. Artificial seawater, prepared in the laboratory using commercially available aquarium salt (19 grams of salt per 0.5 liter of deionized water), was used in the initial evaluation. The stability of LGP in water was initially investigated as part of the analytical method development effort (see Appendix A for additional details to supplement the discussion presented in this section).

Samples of the two water types were prepared in triplicate and were spiked with LGP at concentrations of 2, 10, and 100 times the detection limit for TEAN. This resulted in concentrations of 10.5, 53, and 527 mg/L, respectively. The samples were analyzed after 72 hours of storage at 4°C. Percent recoveries were determined by comparison of the amount recovered to the initial concentration. Analytical interferences were observed in both the groundwater and seawater samples that resulted in consistently high (>100 percent) recoveries of TEAN (Tables 15 and 16, Appendix A). It is speculated that ionic interactions between TEAN and other constituents present in the waters caused a change in detector sensitivity to TEAN. This conclusion was based on the observation that TEAN recoveries from analytical standards which were prepared in deionized water appeared to be randomly distributed around the known concentration. Conversely, measured concentrations of HAN in the groundwater samples were lower than anticipated, especially at lower concentrations. A similar trend was also noted during analysis of the seawater samples. HAN was not recovered from samples spiked with 6.4 and 32.3 mg/L (Table 16, Appendix A). These observations suggest that HAN is less stable than TEAN in aqueous matrices, which is confirmed by previous research reported in the literature.[3] Furthermore, HAN appears to be less stable in seawater than in fresh water.

5.2 *Experimental Design and Methods*

Subsequent to the preliminary assessment of the stability of LGP in aqueous matrices which was conducted during the analytical method development effort, a series of laboratory tests were conducted to acquire more information on the fate of LGP in water. The various matrices included in this evaluation are specified in Table 6. As indicated in the Test Plan, the aqueous matrix used was DI water and DI water with soil to simulate groundwater. The water/soil slurries were 95 percent water and 5 percent soil. Deionized water was used to reduce the analytical interferences.

Each treatment was prepared, in duplicate, in 1.0 liter glass vessels. The total volume of water and soil was 1 L for each treatment. In addition to the soil, each vessel was amended with 500 mg/L RESTORE® and 800 mg/L LGP (152 mg/L TEAN and 488 mg/L HAN). The addition of LGP to the water acidified the samples. The pH of the amended samples was adjusted to between 7.0 and 7.5 by the addition of sodium hydroxide. A mixed consortia of microbial Cultures Nos. 1, 2, and 3, was added to Treatment Nos. 2, 3, and 4. The concentration of each strain in the consortia was approximately 10^7 CFU/mL. The addition of these cultures to the various treatments resulted in a total concentration of approximately 3×10^7 CFU/mL.

Each treatment was then placed in a temperature controlled, computerized respirometer. Subsamples were collected every other week for analysis of HAN, TEAN, nitrate, nitrite, ammonium, DEA, EA, pH, and microbial density. Nitrate and nitrite were measured since nitrate ions should be released if HAN and TEAN dissociate or biodegrade. Additionally, nitrite is an intermediate formed from nitrate during denitrification. Formation/accumulation of high concentrations of nitrite is a potentially undesirable consequence of LGP biodegradation because nitrite is a priority pollutant for groundwater. Diethanolamine and ethanolamine are also potential by-products of TEAN degradation. Total organic carbon and phosphate were measured at the beginning and end of the study. The inorganic carbon produced during treatment was quantified at the end of the study. The biodegradation test was continued for a period of 8 weeks. During this time, additional nutrient amendments and pH adjustments were not added. The analytical methods used for each test are listed in Table 5.

5.3 Results

During the soil biodegradation test early losses of HAN and TEAN were attributed to physical decomposition or adsorption resulting from contact with fresh soil. However, in the water tests a similar phenomenon was not observed indicating that HAN and TEAN were more stable in the aqueous matrix than in soil. Similarly, HAN and TEAN were observed to be stable in deionized water during method development and testing.

5.3.1 HAN and TEAN Degradation

HAN was observed to be rapidly degraded in the soil-water slurries (Figure 12). In contrast, the degradation rate in water without added soil was much slower. The lack of observable differences in the degradation of HAN among the soil-water slurry treatments suggests that the loss of HAN was primarily due to physical or chemical processes rather than biodegradation or adsorption. In the water only (i.e., no added soil) treatment (Treatment No. 3), HAN persisted for a longer time than other treatments. The zero-order degradation rate for HAN was calculated for each treatment regime (Table 7) using the equation shown below:

$$y = ax + b$$

where:

y = measured concentration (mg/L)
x = time (days)
a = slope of concentration vs. time (mg/L per day)
b = y intercept

TEAN concentration in the water sample that did not include added soil (Treatment No. 3) remained essentially unchanged over eight weeks of treatment (Figure 12). Little or no TEAN degradation was observed during the tests. Zero-order degradation rates were calculated for each treatment with the exception of the water-only treatment (Table 7). TEAN degradation rates were observed to be about 100 times less than those observed for HAN degradation.

5.3.2 Microbial Population Size

The bacterial density in each treatment was determined every other week. As seen in Figure 13, the size of the bacterial population increased in Treatment Nos. 1 and 4 by as

much as 100,000 CFU/mL during the test. The size of the bacterial population increased slightly or remained constant in Treatments Nos. 2 and 3. The increase in bacterial density observed in Treatment No. 3 (water-only) did not correspond to a decrease in TEAN. This suggests that bacterial growth was not dependent on TEAN biodegradation. This observation suggests that the presence of a relatively large, growing bacterial population had no affect on TEAN biodegradation.

The presence of soil appeared to be the most important factor associated with the observed decrease in TEAN concentration. The presence of bacteria in the soil does not appear to stimulate TEAN degradation. This conclusion is based on the observation that the treatment including autoclaved soil, which had a much lower initial bacterial density, resulted in the same decrease in TEAN as treatments containing native soil.

5.3.3 Nitrate and Nitrite Concentrations in Water

Nitrate concentrations increased slightly over the course of the study (Figure 14). The measured nitrite concentrations suggest that a small amount of the nitrate was being reduced to nitrite (Figure 14). Nitrite was apparently further consumed since the concentration reached an equilibrium concentration of 10 to 16 mg/L. High levels of nitrite did not accumulate in any of the treatments. There were no obvious trends in the nitrate and nitrite concentration data that distinguished one type of treatment from the others.

5.3.4 Ethanolamine and Diethanolamine Concentrations in Water

EA and DEA were analyzed to determine if they were intermediate products of LGP degradation (Figure 15). Ethanolamine was detected in all samples on the third week of the study; however, this observation was transient and ethanolamine was not detected subsequently. Diethanolamine was also detected on the third week of the study. Diethanolamine concentrations fluctuated between 4 and 14 mg/L from week three through the end of the study five weeks later.

5.3.5 Organic Carbon Mineralization and Respiration

The total organic carbon content of each treatment was evaluated as an indicator of TEAN biodegradation. Because TEAN was the only organic compound added to the treatment vessels, changes in TOC should reflect changes in TEAN if it was being degraded to carbon dioxide. TEAN is 34 percent carbon; therefore, each treatment received an equivalent of

52 mg/L of TOC as TEAN at the initiation of the test. Between 50 and 60 mg/L TOC was detected in each treatment at the beginning of the test period and no appreciable loss of TOC was observed (Figure 16).

Examination of the amount of carbon dioxide (CO_2) produced during treatment revealed that Treatment No. 1 generated about 7.7 mg of CO_2, Treatment No. 2 generated about 7.4 mg of CO_2, Treatment No. 3 generated only 1.25 mg of CO_2, and Treatment No. 4 generated 5.5 mg of CO_2. These are very modest amounts, however, the trend for soil-water slurries to liberate more CO_2 than the water treatment may indicate that some TEAN was being mineralized at a very slow rate. This conclusion is consistent with the TEAN degradation rates presented in Table 7 and Figure 12. It was not possible, because of the low rates, to measure the rate of oxygen consumption in each treatment. The low rates of TEAN degradation and the small amounts of CO_2 produced suggest that the bacterial respiration rate was too low to measure with the respirometers.

5.3.6 Nutrient Utilization and pH

Ammonium was utilized at the same rate and to the same extent in all treatments (Figure 17). The change in ammonium concentration generally correlates with the microbial population density. Phosphate concentrations were slightly higher in samples taken after treatment than before (Figure 18). Phosphate was added to the treatments (in the nutrient solution). A potential explanation for this observation lies in the nature of the complex chemical interactions between phosphate and divalent cations, such as calcium and magnesium. Soluble phosphate and insoluble phosphate-calcium complexes reach an equilibrium that is dependent upon pH. Acidic pH tends to dissociated the phosphate-calcium complexes. The pH of the treatments declined slightly during the investigation which could contribute to an increase in the soluble phosphate concentrations. Additionally, there are other factors that can influence the concentration of soluble phosphate. For example, phosphate can adsorb to soil by a process known as ligand exchange or anion adsorption. This process can be influenced by the presence of other ions, especially oxidizing ions (e.g., hydroxyl ammonium ion).

The initial pH was adjusted to 7.0 to 7.5 after addition of LGP. As indicated in Figure 19, the pH was not stable in all treatments and drifted above pH 7.5 for treatments containing

soil. However, throughout the test, the pH remained between 6.8 and 8.0. A range which is suitable for bacterial growth.

5.4 Summary

The results from the water treatment tests indicate that low concentrations of LGP can be partially treated in water and soil-water slurries simulating groundwater treatment. The results from the aqueous matrix biodegradation test are summarized below:

- HAN disappeared rapidly from water treatments which were amended with soil. The rate of HAN reduction in water treatments without soil was at least 100 times slower.
- Some decomposition of TEAN appears to occur at a low rate when TEAN is in a soil-water slurry.
- Microbial growth did not appear to contribute to TEAN biodegradation.
- Nitrate concentrations were essentially constant during the test, nitrite accumulated to a concentration of 10 to 16 mg/kg in the treated soil.
- Low levels of ethanolamine and diethanolamine were detected, ethanolamine was transient appearing only at 3 weeks, diethanolamine also appeared at 3 weeks and persisted through the rest of the study.
- Total organic carbon in most of the soil-water slurry treatments decreased slightly, the water only treatment was unchanged.
- Low levels of carbon dioxide were generated in the soil-water slurry treatments.
- A decrease in the ammonium concentration generally coincided with bacterial growth and TEAN loss. The decrease in the ammonium concentration occurred at the same rate in all treatments.
- Phosphate assimilation was not observed in any treatment.

6.0 LGP Biodegradation in a Sequencing Batch Reactor

The third phase of the laboratory test program involved the use of bioreactors to continue the evaluation of biodegradation of LGP. Specifically this phase of study was conducted to examine the applicability of bioreactors as a potential treatment technology for LGP in aqueous waste streams. If successful, this approach could provide a practical and mobile technology for treating dilute LGP waste generated during the manufacture, testing, and use of LGP.

Sequencing batch reactor (SBR) technology was used for this test. Operational parameters considered during this bench-scale investigation included hydraulic retention time (HRT), biological solids retention time (BSRT), inoculum development, pH, carbon removal efficiency, total suspended solids (TSS), and mixed liquor volatile suspended solids (MLVSS) concentrations. Each of these parameters is useful for defining the performance of a bioreactor.

6.1 SBR Technology Description

SBR technology was developed in the 1970's from a modification of the "fill and draw" activated sludge process. The "fill and draw" process consists of removing a measured volume of treated waste from the system and introducing a equal volume of influent waste. An SBR system operates similarly to a conventional activated sludge system, however, aeration and solids/liquid separation is accomplished in one tank. This results in reduced capital cost and simplified operation.

The primary control parameters for SBRs are biological solids retention time (BSRT) and hydraulic retention time (HRT). BSRT is the average time a unit of biomass remains in the treatment system. The HRT is the time required to replace one volume of reactor contents. By controlling the BSRT, the specific growth rate and the physiological state of the organisms can be controlled in the reactor. The age of the biomass influences the biodegradation of recalcitrant compounds.

6.2 Test Objectives

The bench-scale treatability study had the following objectives:

- Define control parameters, i.e., BSRT and HRT required for the treatment of an LGP waste stream
- Determine the extent to which LGP will biodegrade in an SBR
- Evaluate the effect of LGP on microbial population density measured as mixed liquor volatile suspended solids (MLVSS)

6.3 Experimental Design and Procedures

Three 1-liter (L) bench-scale glass reactors were used during the bioreactor treatability study (L. H. Fermentation 500 Series III, Stoke Poges, Bucks). All components that contacted reactor contents were glass or stainless steel. Hydrocarbon-free compressed air was used to supply oxygen to the bioreactors through an air sparger installed at the bottom of the reactor. Each reactor was equipped with a low speed agitator to improve the oxygen transfer efficiency. Removal of reactor mixed liquor (wasting) was performed manually. Influent and effluent feed flows were controlled using peristaltic pumps. All pumping, aeration, agitation, and decanting functions were executed by automatic timers. The influent feed container and effluent containers were chilled to minimize chemical and biological degradation prior to analysis. Grab samples of the mixed liquor were extracted via a sampling port located at the top of the reactor. The pH was maintained between 6 and 8 by the manual addition of 1 N NaOH to the reactors.

Each of the three reactors was maintained at a predetermined BSRT set point. The BSRT set points used were 5, 10, and 20 days. These are relatively standard set points that are commonly used for evaluating performance of SBRs. Each reactor cycled through timed phases with two 12-hour cycles per day. The phases and cycle times were consistent for each reactor, while the flow rates were dependent on the BSRT set point. Each cycle consisted of a react-fill cycle during which the aerator and mixer were operating and influent feed was pumped to the reactors for 150 minutes. During the react phase, which lasted 480 minutes, the impeller and aerator operated while there was no flow into or out of the reactor. Following the react phase, the impeller and aerator were turned off and the contents of the reactor were allowed to settle for 60 minutes. During this phase, solids settled producing a clarified liquid and a settled sludge. A decant phase then followed. With the impeller and aerator switched off, the clarified supernatant was decanted for 30 minutes. The effluent flow rate was varied to balance the waste activated sludge (WAS) flow. A high

WAS flow was matched with a low effluent flow. Finally, sludge wasting completed one cycle. The next cycle began again with the mixed fill phase.

Each reactor was operated at the BSRT set point for a period of four sludge ages. The first sludge age was used to obtain steady state conditions; data was collected during the final three ages. The cycle times and flow rates for each reactor are summarized in Table 8. All three reactors were operated at an HRT set point of 2 days. This set point was selected based on the recalcitrant nature of TEAN observed during initial soil and water testing. The concentration of the influent feed was based on the results obtained during activated sludge acclimation (Section 6.7.1.). All process set points were held constant during treatment.

The SBR laboratory scale investigation lasted for a total of 72 days. Each reactor was charged with 750 ml of acclimated activated sludge and the microbial consortia used in the aqueous matrix biodegradation tests (Section 5.0). This consortia consisted of a combination of the organisms which had the highest tolerance to LGP in the screening study. These microbes were used because they had been acclimating to LGP throughout the study period. It was believed that using these acclimated microbes, in addition to the microbes present in the activated sludge, would maximize the probability that microbes capable of degrading LGP would be present. Design parameters for the bench-scale system were as follows:

Reactor 1 - 20-day BSRT

- Feed flow — 1.51 mL/min ± 0.164 mL/min
- HRT — 2.23 days ± 0.23 days
- Air flow — 1 to 10 mL/min
- Temperature — 20°C
- Dissolved oxygen — 3.9 - 10.2 mg/L (avg. 7.4 mg/L)
- pH — 6 - 8
- Agitation — 250 rpm
- BSRT — 19.98 days ± 4.56 days
- Reactor volume — 1 L
- Waste activated sludge — 0 to 0.525 mL/min

Reactor 2 - 10-day BSRT

- Feed flow — 1.52 mL/min ± 0.165 mL/min
- HRT — 2.21 days ± 0.23 days

- Air flow — 1 to 10 mL/min
- Temperature — 20°C
- Dissolved oxygen — 6 - 9.6 mg/L (avg. 7.6 mg/L)
- pH — 6 - 8
- Agitation — 250 rpm
- BSRT — 10.6 days ± 3.02 days
- Reactor volume — 1 L
- Waste activated sludge — 0.325 to 1.875 mL/min

Reactor 3 - 5-day BSRT

- Feed flow — 1.62 mL/min ± 0.18 mL/min
- HRT — 2.08 days ± 0.22 days
- Air flow — 1 to 10 mL/min
- Temperature — 20°C
- Dissolved oxygen — 7.7 - 10.6 mg/L (avg. 9.0 mg/L)
- pH — 6 - 8
- Agitation — 250 rpm
- BSRT — 4.96 days ± 0.49 days
- Reactor volume — 1 L
- Waste activated sludge — 1.225 to 2.5 mL/min

The solids concentration in the reactors was less than 1000 mg/L, which was the minimum concentration desired for initial operation. Therefore, the reactors were spiked with fresh activated sludge. The fresh activated sludge was allowed to settle and the supernatant was withdrawn. Settled solids (250 mL) were placed into each reactor. This brought the solids level to 1,550, 1,790, and 1,630 mg/L for Reactors 1, 2, and 3, respectively.

Approximately 8 L per week of influent feed was required to sustain a 2 day HRT in all reactors. One 20-L carboy was used to supply feed to all three reactors. After one week of operation a 60 to 90 percent loss of solids was observed in the reactors; therefore, a carbon source was added to the feed to sustain the biomass. During the second week of operation, the influent feed consisted of 400 mg/L LGP, 10 mg/L ammoniacal nitrogen, and 50 mg/L ethanol, with the pH adjusted to 7 to 7.5 with 1N NaOH.

Due to the inhibitory effect of LGP on microbes, the HRT was not reduced to 1 day as would be normal practice to provide more carbon to sustain biomass growth, instead a supplemental carbon source (50 mg/L ethanol) was added to the feed.

The effluent flow rate was calibrated daily by weighing the effluent volume removed from each reactor. The influent flow was calibrated to match the combined effluent and WAS volumes.

6.4 Data Analysis

The following equations describe the relationships used to evaluate SBR performance. The BSRT of the bench-scale system was maintained through mass balance of the system solids.

$$BSRT = \frac{XV}{Q_W X_W + (Q - Q_w) X_e} \qquad \text{(Equation 1)}$$

X - TSS in the aeration vessel (mg/L)
V - Volume of the aeration vessel (L)
Q_w - Waste activated sludge (WAS) flow rate (L/day)
X_w - TSS concentration in WAS (mg/L)
Q - Influent flow rate (L/day)
X_e - TSS concentration in system effluent (mg/L).

To maintain the appropriate BSRT set point for each reactor, the Q_w was adjusted. The TSS of the effluent, reactor, and WAS was used to calculate revised Q_w flow rates using Equation 1 above. The Q_w was adjusted twice a week.

The minimum BSRT required for operation without failure can be calculated from the following equation. The calculated value is typically increased using a safety factor of 10 to determine the minimum BSRT for operation.

$$\frac{1}{minimum\ BSRT} = \frac{YkS_o}{K_s + S_o} - K_d \qquad \text{(Equation 2)}$$

Y - Sludge yield coefficient (mg/mg)
k - Maximum substrate utilization rate ($days^{-1}$)
S_o - Influent substrate concentration (mg/L)
K_s - Saturation constant (mg/L)
K_d - Decay rate (day^{-1}).

The HRT of the bioreactor was mathematically determined by dividing the volume of the reactor by the influent flow rate.

The maximum substrate utilization rate was determined using the following equations:

$$q = \frac{kS}{K_s + S} \qquad \text{(Equation 3)}$$

q - Substrate utilization rate ($days^{-1}$)
k - Maximum substrate utilization rate ($days^{-1}$)
S - Substrate concentration surrounding the biomass (mg/L)
K_s - Saturation constant (mg/L)

$$q = \frac{(S_o - S_e)\ Q}{VX} \qquad \text{(Equation 4)}$$

S_o - Influent substrate concentration (mg/L)
S_e - Effluent substrate concentration (mg/L)
Q - Influent flow rate (L/day)
V - Volume of the reactor vessel (L)
X - TSS concentration in the reactor vessel (mg/L)

The food to microorganism ratio (F:M) was determined using the equation presented below.

$$F{:}M = \frac{S_o Q}{VX} \qquad \text{(Equation 5)}$$

The variable observed yield calculated for each BSRT set point was derived from the following equation:

$$Y_{obs} = \frac{Y_{MAX}}{1 + BSRT(K_d)} \qquad \text{(Equation 6)}$$

Y_{obs} - Variable observed yield (mg/mg)
Y_{MAX} - Maximum sludge yield (mg/mg)
K_d - Decay rate (day^{-1})

Equations 1 through 6 were derived from equations presented by Benefield and Randall.[7]

In addition to the maximum substrate utilization rate constant, other biokinetic constants such as the yield coefficient, decay coefficient, and the specific substrate utilization rate constant were determined through graphical analysis of the data.

6.5 Sampling and Analysis

Grab samples of the mixed liquor (suspended reactor contents) was collected directly from each reactor during the react phase. A 12-hour composite sample of the effluent was taken from each reactor. The composite sample was chilled during sampling to reduce biological activity. WAS samples were withdrawn manually from the bottom of the reactor (i.e., sludge blanket) during the decant phase. A single chilled influent container was used to feed all reactors and was sampled weekly for HAN, TEAN, Total Organic Carbon (TOC) and Total Inorganic Carbon (TIC). Table 5 presents a summary of the analytical methods used during the bioreactor test.

The acclimated activated sludge and microbial consortia used for inoculation was analyzed for total Kjeldahl nitrogen (TKN), ammoniacal nitrogen, ortho-phosphate, TOC, COD, BOD, TSS, and MLVSS (Table 9). The activated sludge inoculum had medium to small flocs with moderate interfloc turbidity. An aliquot of the influent feed was analyzed for TOC, COD, BOD, ortho-phosphate, ammoniacal nitrogen, and nitrate, and nitrite (Table 9). Average influent feed concentrations for HAN, TEAN and TOC are shown in Table 10.

The mixed liquor of each reactor was analyzed twice per week for TSS, MLVSS, pH, and dissolved oxygen and weekly for bacterial density. (Due to a contaminated QA sample during the fifth week, the microbial density data for that week was not used.) The mixed liquor was tested twice per week for TSS and MLVSS and bi-weekly for nitrate and nitrite. The effluent was tested weekly for HAN, TEAN, TOC, ammoniacal nitrogen, ortho-phosphate; biweekly for nitrate and nitrite; and twice per week for TSS and MLVSS. The influent feed was tested once per week for TOC, HAN, and TEAN. Because the mixed liquor in an SBR is equivalent to the effluent, most of the analyses were conducted on the effluent stream only to conserve reactor contents.

The volume of the influent, reactor contents, effluent, and WAS was monitored daily. Sample volumes amounted to approximately 20 mL removed every two weeks, which did not significantly affect the reactor volume. Ten mL of deionized water was added to each reactor on two occasions and 40 mL deionized water was added to Reactor 1 once to compensate for evaporation and sample loss.

6.6 QA/QC/Data Management

During the bioreactor test program, all correlation coefficients for calibration curves were greater than 0.95 and all check standards were within ± 10 percent. Matrix spikes and standard additions were prepared at a frequency of 10 percent. Matrix spikes were prepared by spiking the sample to a known concentration, allowing the sample to incubate for one hour, filtering the sample, and then analyzing by HPLC. Standard additions were prepared by filtering a sample then spiking the filtrate to a known concentration and analyzing immediately by HPLC. All spikes and blanks were analyzed under the same conditions as samples. Blanks (deionized water) were included with each sample batch. All spikes and standard additions were within ± 10 percent of the expected value. Table 11 presents QA/QC results.

6.7 Results and Discussions

6.7.1 Activated Sludge Acclimation

The soil and water matrix tests (Sections 4 and 5) demonstrated that TEAN was not readily biodegradable. A long acclimation period and the addition of an easily degradable carbon source was required to culture microbes that could grow in the presence of LGP (Section 2.0). A 4 to 5 week lag phase was observed in the soil matrix test during which time microbial growth occurred before biodegradation of TEAN (Section 4.0). Therefore, the activated sludge, collected from a local publicly owned treatment works (POTW) and augmented with the microbial consortia tolerant to LGP, was acclimated (over a period of three weeks) to increasing concentrations of LGP before reactor start-up.

Activated sludge augmented with the microbial consortia was subjected to two treatments, one with LGP and one with LGP and 2000 mg/L nutrient broth. The nutrient broth was added to supply a complex supplemental carbon source. Earlier in the study, it was

observed that a complex carbon source was required to sustain microbial growth in the presence of LGP.

Additional acclimation time (one sludge age) occurred because the first sludge age of each SBR test was not included in the data collection period. Thus, a minimum of 4 weeks of acclimation time occurred prior to initiation of each test. Four 4-L flasks were filled with activated sludge at a solids density ranging from 1,580 to 2,190 milligram per liter (mg/L) TSS. The consortia of LGP tolerant bacteria was added to each flask to a final density of 10^7 CFU/mL. Two of the flasks were spiked to a final concentration of 100 mg/L LGP and 2,000 mg/L nutrient broth. The other two flasks were spiked to a final concentration of 100 mg/L LGP. Ammoniacal nitrogen and pH were analyzed weekly and maintained at or above 10 mg/L and between 6 and 8, respectively. Data obtained during the acclimation phase is presented in Table 12.

Each flask of sludge was supplemented to 400 mg/L LGP during the second and third weeks of the acclimation period. Because sludge loss was observed, acclimation with a higher concentration of LGP was not attempted. The treatments that contained nutrient broth were supplemented once a week with 2,000 mg/L nutrient broth. After the end of the third week, the treatment with nutrient broth had a greater concentration of solids.

A microbial evaluation of the two treatments at the end of the third week of acclimation indicated that nutrient broth resulted in high interfloc turbidity, interspersed growth, and small flocs that appeared to be breaking up. The treatments without nutrient broth contained medium flocs with less interfloc turbidity. Based on these results, it was determined that sludge cultures acclimated with nutrient broth would settle poorly and wash out of the reactor. Therefore, the cultures acclimated without supplemental nutrient broth were used in the SBR tests.

6.7.2 SBR Performance Results

Residual concentrations of 5 mg/L ammoniacal nitrogen and 1 mg/L ortho-phosphate typically result in balanced microbial growth.[7] These residual concentrations were targeted during the reactor investigation by bringing the influent feed to a concentration of 10 mg/L NH_4+ as NH_4CL. Ammoniacal nitrogen and ortho-phosphate concentrations are presented

in Tables 13, 14, and 15. The TOC removal efficiencies were determined throughout the course of the investigation at each BSRT (Figure 20).

The influent HAN and TEAN concentrations averaged 273 mg/L and 107 mg/L, respectively. The effluent concentrations of HAN and TEAN for each BSRT set point are presented in Tables 13, 14, and 15. HAN removal efficiency is presented in Figure 21. The removal efficiency of TEAN (Figure 22) was negligible for all three reactors throughout the study.

The TSS and MLVSS concentrations at each sludge age are presented in Tables 13, 14, and 15. Review of the TSS concentration at each BSRT and sludge age illustrates the loss in solids during operation (Figure 23). This loss of biomass is also evident in Figure 24, the MLVSS concentration versus BSRT, and Figure 25, the MLVSS:TSS ratio versus BSRT. The loss of biomass was observed to be more rapid with decreasing BSRT. Each reactor was maintained at three sludge ages (5, 10 and 20 days).

20-day BSRT

One SBR was evaluated under steady-state conditions with a 20 day BSRT for a total of 61 days. Data obtained during this period of operation are summarized in Table 13. The BSRT and HRT averaged 19.98 and 2.23 days, respectively. The effluent and sludge wasting flow rates which were maintained to establish the 20-day BSRT averaged 7.47 mL/min and 0.199 mL/min, respectively. The influent flow rate averaged 1.50 mL/min.

The influent TOC concentrations averaged 59.3 mg/L and effluent concentrations averaged 46.6 mg/L, a removal efficiency of 21.3 percent. During the first, second, and third sludge age, the effluent TOC concentrations were 43.2, 38.8, and 57.5 mg/L, respectively. The average F:M ratio based on TOC concentrations during this period was 0.042 $days^{-1}$.

The influent concentrations of HAN and TEAN averaged 273 mg/L and 107 mg/L, respectively. The percent removal of HAN averaged 72.6 percent while there was negligible reduction in TEAN concentrations during the course of the study. The removal efficiencies for HAN during the first, second, and third sludge ages were 79.9, 85.9, and 52.8 percent, respectively.

The mixed liquor TSS and MLVSS concentrations averaged 634 and 392 mg/L, respectively. During the first, second and third sludge age the TSS concentration in the reactors averaged 979, 778, and 151 mg/L, respectively. The MLVSS concentration during the same periods averaged 616, 443, and 119 mg/L, respectively. The TSS and VSS for WAS averaged 3915 and 2640 mg/L, respectively. The TSS was 6790 and 5000 mg/L for the first and second sludge age, respectively. The MLVSS for the first and second sludge age was 4700 and 3240 mg/L, respectively. Sludge was not wasted after the second sludge age due to the loss in biomass. The TSS and VSS for the effluent averaged 46.5 and 24.3 mg/L, respectively. The TSS in the effluent for the first, second, and third sludge ages were 66, 54, and 19 mg/L, respectively. The MLVSS in the effluent was 39, 21, and 13 mg/L for the first second and third sludge age, respectively. The MLVSS:TSS ratio averaged 64 percent. Based on TOC, the amount of biomass produced was 5.2×10^{-6} lb/day.

The ammoniacal nitrogen concentration in the effluent averaged 9.26 mg/L and ortho-phosphate averaged 3.09 mg/L. This indicates that adequate nitrogen and phosphate were present for microbial activity.

Microscopic examination of the reactor mixed liquor during the 20-day BSRT indicated an increase in interfloc turbidity throughout operation. As the solids were washing out, there was an increase in pin point floc formation and an eventual loss of flocs.

10-day BSRT

The SBR operated at a 10-day BSRT was evaluated over three sludge ages over a period of 30 days. Data generated during this period of operation are presented in Table 14. The effluent flow rate and waste flow rate averaged 7.48 mL/min and 1.03 mL/min, respectively. The influent flow rate averaged 1.52 mL/min.

The average BSRT was determined to be 10.6 days. The average HRT was 2.21 days. The influent waste stream had an average TOC concentration of 55.7 mg/L and the effluent TOC concentration averaged 37.5 mg/L (41.3, 34.1, and 36.6 mg/L during the first, second, and third sludge ages). The TOC removal efficiency averaged 30.6 percent (11.0, 44.5, and 38.8 for the first, second, and third sludge age, respectively). The average F:M ratio based on TOC was 0.038 $days^{-1}$.

The influent concentrations of HAN and TEAN averaged 232 mg/L and 97.2 mg/L, respectively. The percent removal of HAN during the first sludge age was 28 percent, 73 percent over the second sludge age, and 65.5 percent during the third sludge age. The overall percent removal of HAN was 54.6 percent. Significant reductions in TEAN concentration did not occur during the first sludge age and only 12.6 percent and 4.8 percent removal was observed during the second and third sludge ages, respectively.

The average TSS concentration in the reactor was 662 mg/L: 873, 704, and 391 mg/L during the first, second, and third sludge ages, respectively. The effluent TSS concentration averaged 42.5 mg/L: 47.5, 40.0, and 40.3 mg/L, during the first, second, and third sludge age, respectively. The TSS of the waste sludge averaged 5890 mg/L, with a first, second, and third sludge age average of 6340, 7,090, and 4,280 mg/L, respectively. The MLVSS:TSS ratio averaged 52 percent with the first, second, and third sludge age averages at 60, 61, and 35 percent. These data indicate the loss of biomass in the reactor over time, suggesting either toxicity or inhibition of microbial growth.

The effluent ammoniacal nitrogen and ortho-phosphate concentrations averaged 8.5 and 5.5 mg/L, respectively, which is adequate to support microbial activity.

Microscopic examination of the activated sludge flocs revealed changes in floc morphology over time. Initially, medium-sized flocs with low interfloc turbidity existed. The loss in solids led to an increase in interfloc turbidity and pin-point floc formation, and eventually led to a decrease in floc formation.

5-day BSRT

The bioreactor operated at a 5 day BSRT was evaluated over a period of 15 days. Data generated during this bioreactor investigation are presented in Table 15. The BSRT and HRT during this period of operation averaged 4.96 and 2.08 days, respectively. The influent flow rate maintained during this period was 1.62 mL/min, with an effluent flow rate of 7.87 mL/min and a waste sludge flow rate of 0.202 mL/min.

The average F:M ratio based on TOC during this period was 0.060 $days^{-1}$. The influent TOC concentration averaged 51.34 mg/L and TOC removal efficiency averaged 19.5 percent (6.28, 11.1, and 43.8 percent during the first, second, and third sludge age, respectively).

The influent concentrations of HAN and TEAN averaged 209.5 and 90.0 mg/L, respectively. The overall percent removal of HAN was 44.0 percent (11.0, 61.4, and 66.0 percent during the first, second, and third sludge ages, respectively). There was no significant change in TEAN concentration during the first sludge age. During the second and third sludge ages, 15.4 and 13.5 percent TEAN removal was observed.

The mixed liquor TSS and MLVSS concentrations averaged 416 and 209 mg/L, respectively. During the first, second, and third sludge age the TSS concentrations averaged 397, 514, and 340 mg/L, respectively. The MLVSS concentrations averaged 237, 254, and 132 mg/L, respectively, during the same periods. The MLVSS:TSS ratio averaged 45 percent (47, 49 and 39 percent during the first, second and third sludge age, respectively).

The effluent TSS and MLVSS concentrations averaged 25.0 and 11.4 mg/L, respectively. The TSS concentration of the effluent during the first, second, and third sludge age averaged 21.3, 29.2, and 25.2 mg/L, respectively. The MLVSS concentration during these periods averaged 8.93, 9.20, and 16.6 mg/L, respectively.

The TSS and MLVSS concentrations in the WAS averaged 5490 and 3760 mg/L, respectively. The TSS concentration during the first, second, and third sludge age averaged 3720, 7640, 5450 mg/L, respectively. The MLVSS concentration averaged 2600, 5180, and 3740 mg/L during these periods, respectively.

The effluent ammoniacal nitrogen and ortho-phosphate concentrations averaged 6.57 and 6.53 mg/L, respectively.

Microscopic evaluation of the system's activated sludge flocs indicated that small to medium flocs were present at start-up with interfloc turbidity increasing over time. By the third sludge age there were no filaments or flocs. The residual biomass was interspersed.

6.7.3 Biokinetic Constants

The performance of the SBRs in degrading LGP was poor. The BSRT and HRT required for the treatment of LGP could not be determined because each reactor ultimately experienced biomass loss due to the lack of TEAN biodegradation. HAN was effectively degraded; however, TEAN was persistent and passed through each reactor with very little

degradation. The performance of each reactor and the observed loss of biomass over the course of three sludge ages indicated that LGP provided a very poor growth substrate for the biomass under these conditions.

Biokinetic constants derived from measured TOC concentrations are summarized in Table 16. The specific substrate utilization rate constant (K) based on TOC, HAN, and TEAN was calculated. K was derived from the slope of the plot of specific substrate utilization rate (q) vs. BSRT ($days^{-1}$). The small K values indicate the poor performance of the bioreactors.

Overall substrate utilization rates (q) calculated for the 20-day BSRT reactor were 0.011, 0.27, and 0.0126 $days^{-1}$ for TOC, HAN, and TEAN, respectively. The q for the 10-day BSRT reactor based on TOC and HAN were 0.016 and 0.119 $days^{-1}$, respectively. The q based on TEAN was negative due to the negligible removal of TEAN. The negative q value results from calculation using the observed data. It indicates no significant change and is the result of variability in the analytical method; it does not indicate that TEAN was generated. The overall q's for the 5-day BSRT based on TOC and HAN were 0.016 and 0.137 $days^{-1}$, respectively. The maximum sludge yield (Ymax) was 0.18 mg biomass/mg TOC. The observed sludge yields were 0.023, 0.100, and 0.138 mg biomass/mg TOC for 20, 10, and 5 day BSRT reactors, respectively. The microorganism decay rate could not be accurately calculated because of lack of growth and biomass loss by wash out.

The average q values calculated for each BSRT set point during each sludge age are presented in Table 16. (The biokinetic constant calculations are contained in Appendix C.) Calculation of a minimum BSRT requires the substrate utilization rate for TOC, the sludge yield, and the microorganism decay rate. However, the minimum BSRT could not be determined because TEAN was not biodegraded and TOC reduction occurred only when ethanol was added. Additionally, an accurate microorganism decay rate could not be calculated because biomass was not sustained in the reactors. The minimum BSRT must be greater than 20 days since all three reactors experienced biomass washout.

7.0 Conclusions and Recommendations

7.1 Conclusions

Based on the observations and findings resulting from the laboratory investigations reported in the previous sections (and Appendices) of this report, the following conclusions have been drawn:

- LGP is toxic or inhibitory to soil microbes. Although the specific threshold for toxic or inhibitory effects was not determined, 100 ppm of LGP did not appear to have an effect while concentrations of 1000 ppm inhibited all microbial growth.

- Bacteria isolated during the investigation that were tolerant to LGP at concentrations up to 800 ppm LGP were aerobic, gram negative soil bacteria: two cultures were identified as Pseudomonas sp., the third was not identified.

- LGP could not be used by microbes as the sole source of carbon and energy. Nutrient broth was a required supplement for bacterial growth in the presence of LGP. Simple carbon sources such as glucose, acetate, and methanol did not support bacterial growth in the presence of LGP.

- The HPLC analytical method, referred to herein as the USAWES method, allows the quantification of HAN and TEAN at low levels (i.e., <10 ppm) in environmental samples. However, this method is labor intensive, requires a skilled analyst, and can be affected by matrix interferences. Other methods documented in the literature were found to be inadequate because they cannot achieve low detection limits.

- HAN was observed to be rapidly degraded in soil and aqueous matrices. The mechanism, although not defined, is believed to be chemical/physical as opposed to biological. (This finding is consistent with previous research by Kaplan.) In tests using SBR's with BSRT's of 5, 10, and 20 days, HAN reductions of 44, 55, and 73 percent were observed.

- A significant degradation of TEAN was observed in soil tests that was correlated with biological activity. There was no apparent difference in biodegradation by native and LGP tolerant microbes. This implies that in situ land treatment could be a viable remediation technology if LGP concentrations are not inhibitory to microbes.

- TEAN was observed to be recalcitrant to degradation during testing in aqueous matrices and in sequencing batch reactors operated at 5 to 20 day BSRTs. Changes in TEAN concentrations did not exceed the limits of analytical variability.

- When added to soil or aqueous samples, LGP acidifies the matrix. Correction to neutral pH was required during the laboratory testing.

- LGP tolerant strains did not enhance biodegradation of HAN or TEAN.

7.2 Recommendations

Based on the observations, findings and conclusions resulting from the laboratory investigations documented in this report, the following recommendations are made.

A complete validation of the USAWES HPLC analytical method, including interlaboratory studies with round-robin analysis, should be completed. This effort would provide a standard method that could be used by researchers and investigators to quantify low levels of LGP in environmental samples. The identification and resolution of matrix interferences should also be addressed.

A standardized method for extraction of LGP from soils should be developed, fully validated and documented. As LGP is put into field use, the need to quantify impacts from spills will be required.

Further investigations should be directed at determining whether biodegradation of TEAN is feasible. Additional laboratory studies involving a 40 day BSRT could be established in bench-scale, 1 to 5 liter reactors. The reactors could be seeded with LGP acclimated activated sludge obtained from a wastewater plant at a manufacturer of LGP if such a source exists. In the event that sludge cannot be obtained from such a source, sludge from a POTW could be used. The sludge should be acclimated for a maximum of 90 days or until evidence indicating biodegradation of TEAN is observed. Thereafter, the reactor would be operated as an SBR following similar operation and maintenance practices used during the current investigation. Since TEAN has proven to be a poor growth substrate, ethanol could be added to the feed to sustain the biomass. Additionally, a longer anaerobic settling phase should be evaluated to encourage greater denitrification. It is recommended that the reactor test program be conducted over three 40-day sludge ages to insure steady

state operation. If this reactor operating plan does not yield measurable TEAN removal, biological treatment of TEAN in an aqueous waste stream may not be feasible. Alternatively, if TEAN removal is documented, a subsequent step would be to scale up to a pilot-scale demonstration at a Liquid Gun test site to treat residuals generated during field testing of the gun. A successful pilot plant demonstration should provide the data necessary to develop a transportable biological treatment system. For in situ soil treatment, a large scale field investigation or demonstration should be conducted, if the 40 day SBR test is successful, to demonstrate and quantify in situ degradation rates.

8.0 References

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List of Tables

Table 1

Sources of Microbes Used to Acquire LGP Tolerant Strains

Culture Number	Source of Microbes
1	A Known Crude Oil Degrader
2	Mushroom Cultivation Compost
3	Bunker C-Impacted Soil
4	Garden Soil
5	Soil from a Wood-Treating Site
6	Petroleum-Impacted Soil
7	Hydrocarbon-Impacted Soil
8	PAH-Impacted Soil
9	Phthalate-Impacted Groundwater
10	Industrial Wastewater from a Lagoon in California
11	Activated Sludge
12	Anaerobic Sludge
13	Horse Manure
14	Top Soil

Table 2

LGP Degradation by LGP Tolerant Strains

HAN	Actual[1] (mg/L)	Day 0[2] (mg/L)	Day 4 (mg/L)	Day 10 (mg/L)	Day 19 (mg/L)	Day 33 (mg/L)
Strain 1	488	315	155	55	13	0
Strain 2	488	315	162	93	19	0
Strain 3	427	240	87	30	23	0
Strain 5	366	230	149	94	26	0
Strain 6	244	137	38	11	3.8	0
Strain 4	244	137	NS[3]	46	32	0
TEAN	**Actual[1]**	**Day 0[2]**	**Day 4**	**Day 10**	**Day 19**	**Day 33[4]**
Strain 1	152	144	180	139	183	327
Strain 2	152	144	175	132	156	235
Strain 3	133	104	129	118	125	163
Strain 5	114	97	128	105	128	212
Strain 6	76	75	NS[3]	56	68	96
Strain 4	76	75	82	75	83	90

[1]Actual starting concentration determined by volumetric addition of a standard solution.
[2]Day 0 measurements based on analysis of aliquots taken immediately after addition of standard solution.
[3]NS - No sample
[4]Elevated concentrations are due to reduced volume of samples by 30 to 40 percent due to evaporation.

Table 3

Description of LGP Tolerant Cultures Selected for Use

- **Culture 1**
  - crude oil-degrader
  - tolerance to 800 ppm LGP
  - pseudomonas

- **Culture 2**
  - mushroom cultivation compost
  - tolerance to 800 ppm LGP
  - pseudomonas

- **Culture 3**
  - bacteria from a site contaminated with Bunker C fuel oil
  - tolerance to 800 ppm LGP
  - identification unknown - low correlation in microbial tests

Table 4

Descriptions of Soil Matrix Test Treatments

Treatment Number	Description
LT1 and LT2	Clay soil with LGP, Restore[1], and Lime additions
LT3 and LT4	Clay soil with LGP, Restore, Lime, and Culture 1
LT5 and LT6	Clay soil with LGP, Restore, Lime and Culture 2
LT7 and LT8	Clay soil with LGP, Restore, Lime, and Culture 3
LT9 and LT10	Sterile clay soil with LGP, Restore, Lime, and Culture 1
LT11 and LT12	Sterile clay soil with LGP, Restore, Lime and Culture 2
LT13 and LT14	Sterile clay soil with LGP, Restore, Lime and Culture 3

[1]Restore 375® (IT Corporation) is a water soluble microbial nutrient formulated for in situ bioremediation applications. It contains ammonia chloride, mono- and dibasic phosphate and sodium tripolyphosphate.

Table 5

Summary of Analytical Methods

Parameter	Method Number	Method Title	Method Type
HAN/TEAN	--	USA WES Method	HPLC
Ethanolamine/Diethanolamine	--	USA WES Method	HPLC
TOC/TIC	BAC008	Carbon Analysis Using the Dorhmann Total Carbon Analyzer	Persulfate oxidation
TSS	Standard Method 2540 D.	Total Suspended Solids Dried at 103-105°C	Drying oven
VSS	Standard Method 2540 E.	Fixed and Volatile Solids Ignited at 500°C	Drying oven
Ammoniacal Nitrogen	BAC022	Electrometric Ammonia Analysis	Ion probe
Ortho-phosphate	BAC015	Phosphate Analysis	Colorimetric
Total Heterotrophs	BAC009	Microbial Enumerations	Spread plate
Nitrate	--	Isocratic elution of anions	HPLC
Nitrite	--	Isocratic elution of anions	HPLC
BOD	--	5-day BOD	Galvanic cell
COD	EPA 410.1	COD analysis	Digestion
TKN	EPA 351.1	TKN analysis	Digestion
pH	BAC014	pH Analysis	All Electrode
DO	BAC021	Oxygen Analysis	Galvanic cell

Table 6

Descriptions of Soil-Water Matrix Treatments

Treatment	Sterile Deionized Water	Soil	Nutrients	Bacteria	pH Adjusted	LGP
1	950 mL	50 g	500 mg/L Restore	---	✓	800 mg/L
2	950 mL	50 g	500 mg/L Restore	10^7 CFU/mL	✓	800 mg/L
3	1000 mL	none	500 mg/L Restore	10^7 CFU/mL	✓	800 mg/L
4	950 mL	50 g (autoclaved)	500 mg/L Restore	10^7 CFU/mL	✓	800 mg/L

Table 7

Calculated Zero-Order Degradation Rates for HAN and TEAN in Soil Water Slurries

Treatment[1]	Variable	HAN Degradation Rates (mg/L - day)	TEAN Degradation Rates (mg/L - day)
1	Soil	-411	-5
2	Soil, bacteria	-382	-3.5
3	Bacteria	-058	ND[2]
4	Sterile soil, bacteria	-360	-3.8

[1] Treatments defined in Table 6.

[2] Not degraded, examination of Figure 12 indicates variability between duplicate treatments. Visual observation of degradation curves suggest no degradation during the test.

Table 8

Cycle Times and Flow Rates Used During SBR Tests

Cycle (2/day)	Time (minutes)	20-day BSRT Flow rate (mL/min)	10-day BSRT Flow rate (mL/min)	5-day BSRT Flow rate (mL/min)	Influent	Mixer	Aeration	Decant	WAS
React Fill	150	1.51	1.52	1.62	On	On	On	Off	Off
React	480	Off	Off	Off	Off	On	On	Off	Off
Settle	60	Off	Off	Off	Off	Off	Off	Off	Off
Decant	30	7.47	7.48	7.87	Off	Off	Off	On	Off
Sludge Waste[1]	4	0.199	1.03	0.202	Off	Off	Off	On	On

[1]Sludge waste occurred during the decant phase.

Table 9

Initial Sludge Characteristics (mg/L)

Sample	NH4	TKN	PO4	TOC	COD	BOD	TSS	MLVSS	HAN	TEAN	NO2	NO3
Initial charge	22.6	10	39	42.4	170	200	2,590	1,610	NA	NA	NA	NA
Influent feed	11.8	160	< 0.5	33.4	4000	NA	< 10	< 10	230	129	7.5	220

Table 10

Average Influent Feed Parameters (mg/L)

HAN	TEAN	TOC
273	107	59.3

Table 11

QA Sample Results (Percent Recovery ± Standard Deviation)

	HAN	TEAN	Nitrate	Nitrite
Check Standard	98 ± 3.9	102 ± 5.1	100 ± 3.6	99 ± 3.3
Matrix Spikes	94 ± 6.0	101 ± 4.3	101 ± 4.2	91 ± 5.9
Standard Addition	94 ± 6.6	100 ± 9.0	101 ± 8.1	90 ± 8.3

Table 12

Operational Data Obtained During Acclimation of Activated Sludge

Sample	Solids (mg/L)			Ammoniacal Nitrogen (mg/L)			pH		
	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Nutrient Broth - A[1]	2,180	2,090	1,790	149	240	338	7.1	8.0	8.1
Nutrient Broth - B[1]	2,130	1,420	1,810	154	250	338	7.9	7.8	8.7
No Nutrient Broth - A	1,460	580	1,080	< 1.0	19.7	24.5	5.5	7.1	7.4
No Nutrient Broth - B	1,690	840	1,130	< 1.0	21.5	20.6	5.9	6.6	7.5

[1]A and B indicate duplicate acclimation cultures.

Table 13

Operational Parameters for Reactor No. 1: 20 Day BSRT

Parameter	1st Sludge Age	2nd Sludge Age	3rd Sludge Age	Average Total
HRT (days)	2.14	2.34	2.30	2.23
BSRT (days)	21.06	20.75	18.19	19.98
Microbial Density(CFU/g)	1.8E + 07	7.9E + 07	7.3E + 06	3.5E + 07
NH_4 (mg/L)	4.2	11	12.6	9.26
PO_4 (mg/L)	7.1	1.87	0.3	3.09
Nitrite (mg/L)	10.6	10.8	6.3	9.23
Nitrate (mg/L)	210	227	210	216
TSSrx (mg/L)	979	778	151	634
MLVSSrx (mg/L)	616	443	119	392
TSSe (mg/L)	66	54	19	46.5
MLVSSe (mg/L)	39	21	13	24.3
TSSw (mg/L)	6790	5000	0	3920
MLVSSw (mg/L)	4700	3240	0	2640
HAN (mg/L)	33.6	38.4	157	77.0
HAN removal (%)	79.9	85.9	52.8	72.6
q HAN (days-1)	0.0870	0.140	0.573	0.269
TEAN (mg/L)	97.7	102	114	104
TEAN removal (%)	-13.7[1]	5.55	6.80	-0.55
q TEAN (days-1)	-0.0056	0.0035	0.0394	0.0126
TOC (mg/L)	43.2	38.8	57.5	46.6
TOC removal (%)	17.8	33.6	12.8	21.3
q TOC (days-1)	0.0048	0.0122	0.0149	0.0106
MLVSS:TSS (%)	62	56	75	64

[1]Negative removals (i.e., effluent concentration exceeding influent concentration) indicate an analytical artifact possibility due to interferences or variability in the method.

Table 14

Operational Parameters for Reactor No. 2: 10 Day BSRT

Parameter	1st Sludge Age	2nd Sludge Age	3rd Sludge Age	Average Total
HRT (days)	2.21	2.16	2.25	2.21
BSRT (days)	10.8	9.47	11.35	10.6
Microbial density (CFU/g)	2.5E + 07	3.7E + 07	4.3E + 07	3.5E + 07
NH4 (mg/L)	5.3	10.1	10.1	8.5
PO4 (mg/L)	11.5	3.25	1.8	5.52
Nitrite (mg/L)	6.9	7.4	6.4	6.9
Nitrate (mg/L)	175	189	205	190
TSSrx (mg/L)	873	704	391	662
MLVSSrx (mg/L)	555	438	138	382
TSSe (mg/L)	47.5	40.0	40.3	42.5
MLVSSe (mg/L)	22	24	15	20
TSSw (mg/L)	6340	7090	4280	5890
MLVSSw (mg/L)	4139	4920	2674	3897
HAN (mg/L)	104	67.9	90.7	88.3
HAN removal (%)	28.0	73.1	65.5	54.6
q HAN (days-1)	0.0342	0.1282	0.2035	0.1195
TEAN (mg/L)	99.4	92	100	97.4
TEAN removal (%)	-32.6[1]	12.6	4.68	-5.99
q TEAN (days-1)	-0.0192	0.0087	0.0061	-0.0024
TOC (mg/L)	41.3	34.1	36.6	37.5
TOC removal (%)	11.0	44.5	38.8	30.6
q TOC (days-1)	0.0031	0.0196	0.0274	0.0163
MLVSS:TSS (%)	60	61	35	52

[1]Negative removals indicate an analytical artifact possibly due to interferences or variability in the method.

Table 15

Operational Parameters No Reactor 3: 5 Day BSRT

Parameter	1st Sludge Age	2nd Sludge Age	3rd Sludge Age	Average Total
HRT (days)	2.13	2.03	2.06	2.08
BSRT (days)	5.05	4.65	5.17	4.96
Microbial density (CFU/g)	1.8E + 07	2.1E + 07	1.6E + 07	1.8E +07
NH4 (mg/L)	4.3	5.3	10.1	6.57
PO4 (mg/L)	7.6	9.8	2.2	6.53
Nitrite (mg/L)	8.3	6.9	7.2	7.5
Nitrate (mg/L)	216	175	188	193
TSSrx (mg/L)	397	514	340	416
MLVSSrx (mg/L)	237	254	132	209
TSSe (mg/L)	21.3	29.2	25.2	25
MLVSSe (mg/L)	8.93	9.20	16.6	11.4
TSSw (mg/L)	3717	7644	5448	5485
MLVSSw (mg/L)	2600	5180	3744	3764
HAN (mg/L)	105	105	87.4	99.5
HAN removal (%)	11.0	61.4	66.0	44.0
q HAN (days-1)	0.0223	0.1661	0.2455	0.1370
TEAN (mg/L)	120	88.0	92.8	100
TEAN removal (%)	-87.5[1]	15.4	13.5	-23.8
q TEAN (days-1)	-0.0965	0.0158	0.0209	-0.0247
TOC (mg/L)	35.7	47.9	34.1	39.8
TOC removal (%)	11.3	11.1	43.8	19.5
q TOC (days-1)	0.0384	0.0060	0.0408	0.0161
MLVSS:TSS (%)	47	49	39	45

[1]Negative removals indicate an analytical artifact possibly due to interferences or variability in the method.

Table 16

Calculated Biokinetic Constants for TOC, HAN, and TEAN

BSRT	q TOC (day^{-1})	K_s TOC (mg/L-day)
20-day	1.06×10^{-2}	3.0×10^{-4}
10-day	3.4×10^{-2}	3.3×10^{-3}
5-day	8.6×10^{-2}	1.2×10^{-2}
BSRT	**q HAN (day^{-1})**	**K_s HAN (mg/L-day)**
20-day	2.7×10^{-1}	3.8×10^{-3}
10-day	2.25×10^{-1}	5.8×10^{-3}
5-day	5.1×10^{-1}	8.5×10^{-3}
BSRT	**q TEAN (day^{-1})**	**K_s TEAN (mg/L-day)**
20-day	1.25×10^{-2}	2.9×10^{-3}
10-day	4.8×10^{-3}	3.3×10^{-3}
5-day	1.6×10^{-2}	4.7×10^{-3}

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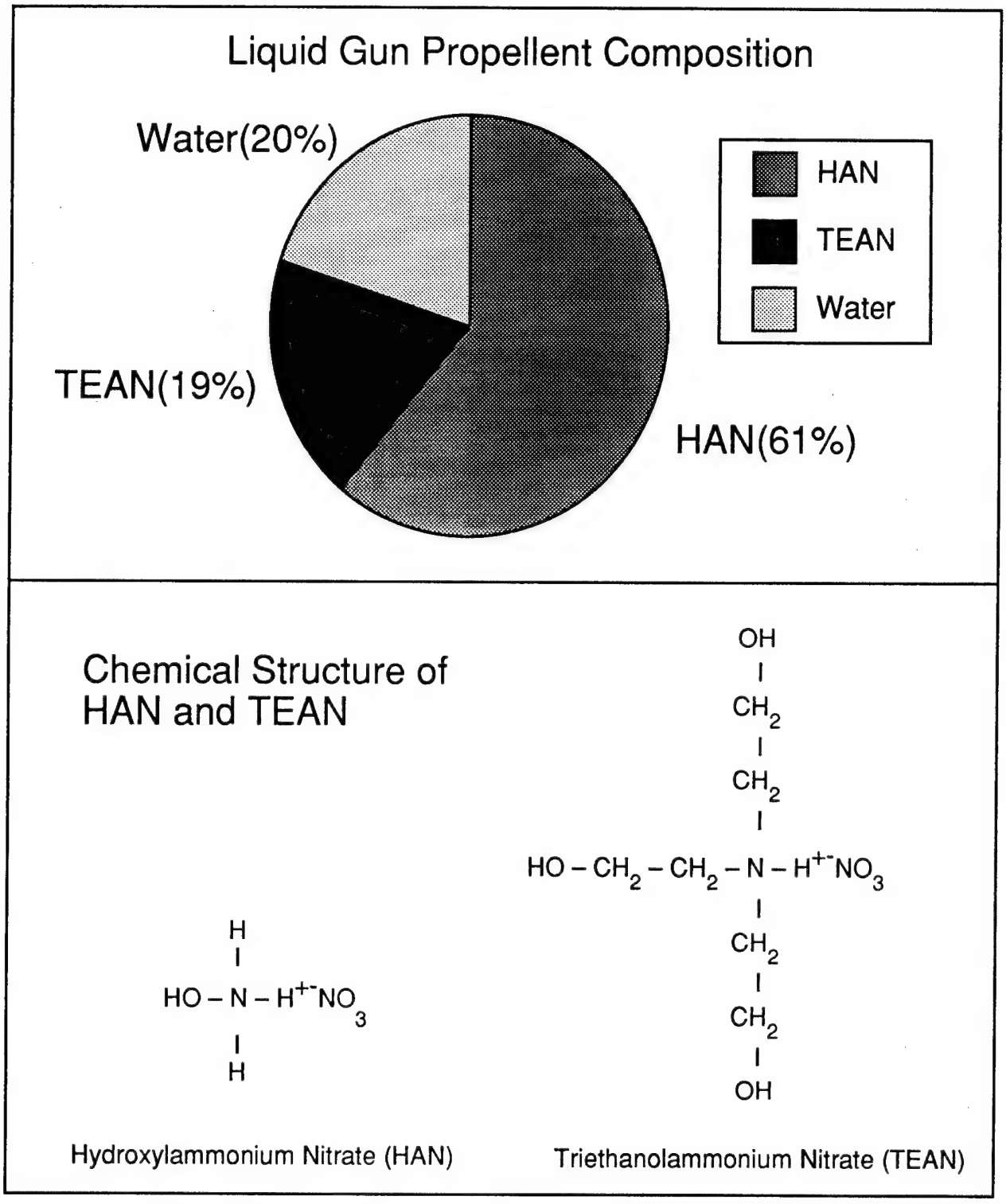


Figure 1.
Composition of LGP and Chemical Structure of HAN and TEAN.

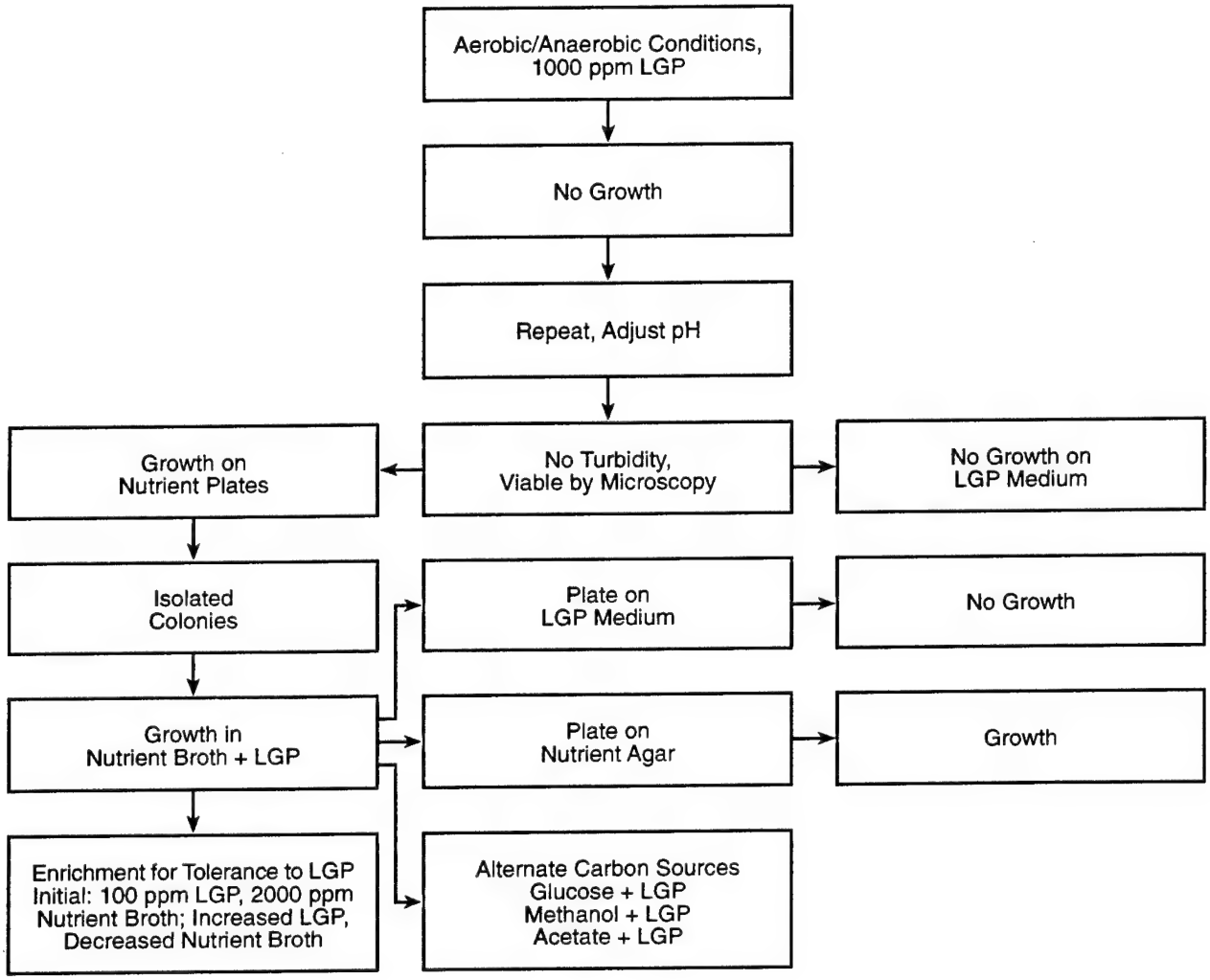


Figure 2.
Microbial Enrichment and Selection.

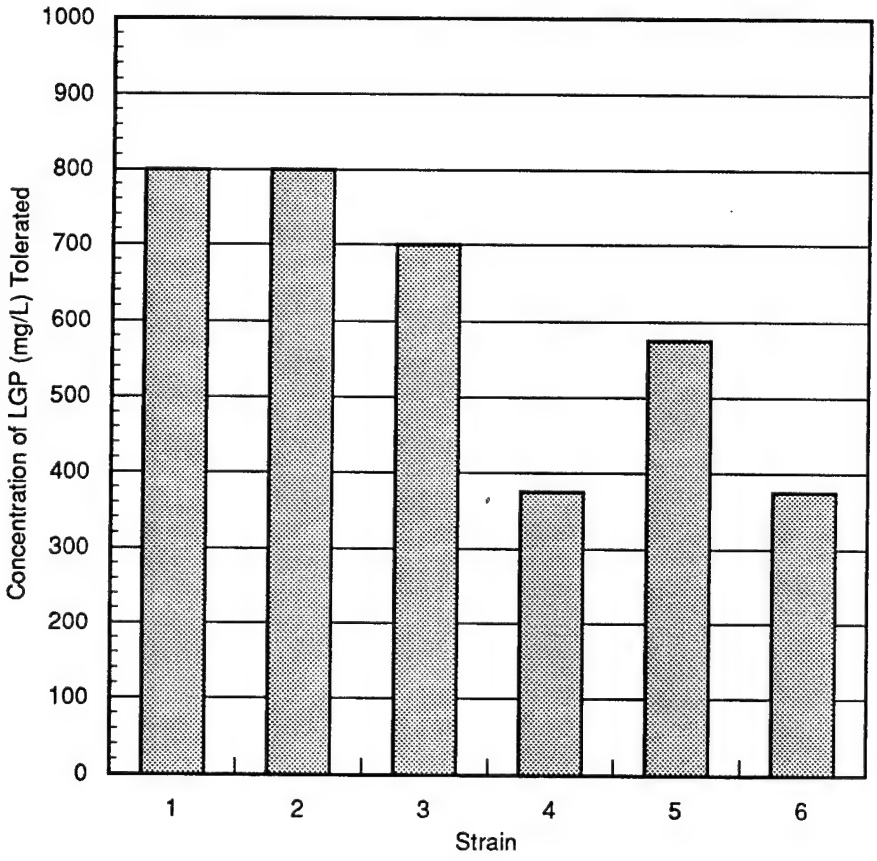


Figure 3.
Tolerance of Selected Bacterial Strains to LGP.

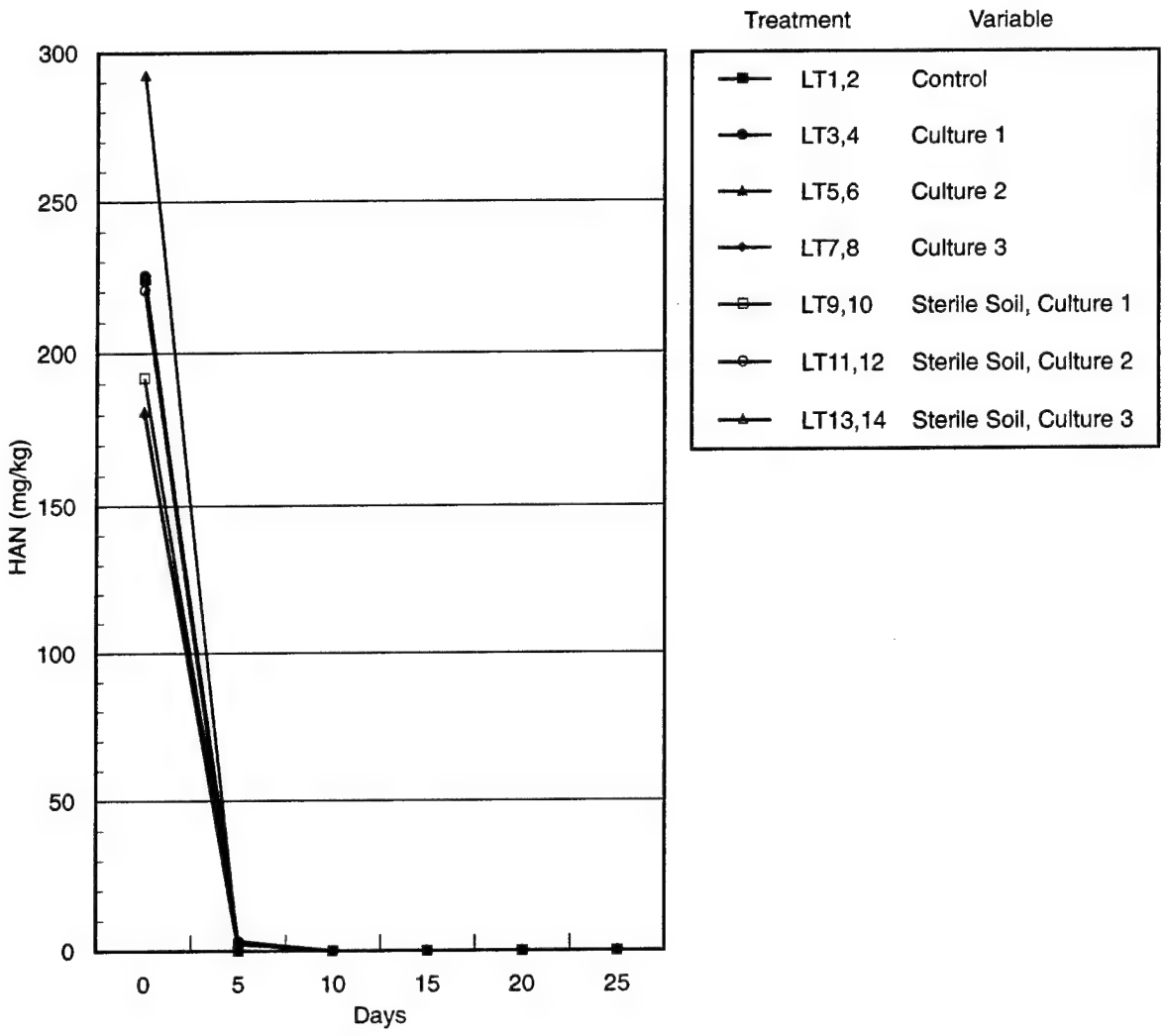


Figure 4.
HAN Degradation Observed During Soil Matrix Test.

(soil treatments as defined in Table 4)

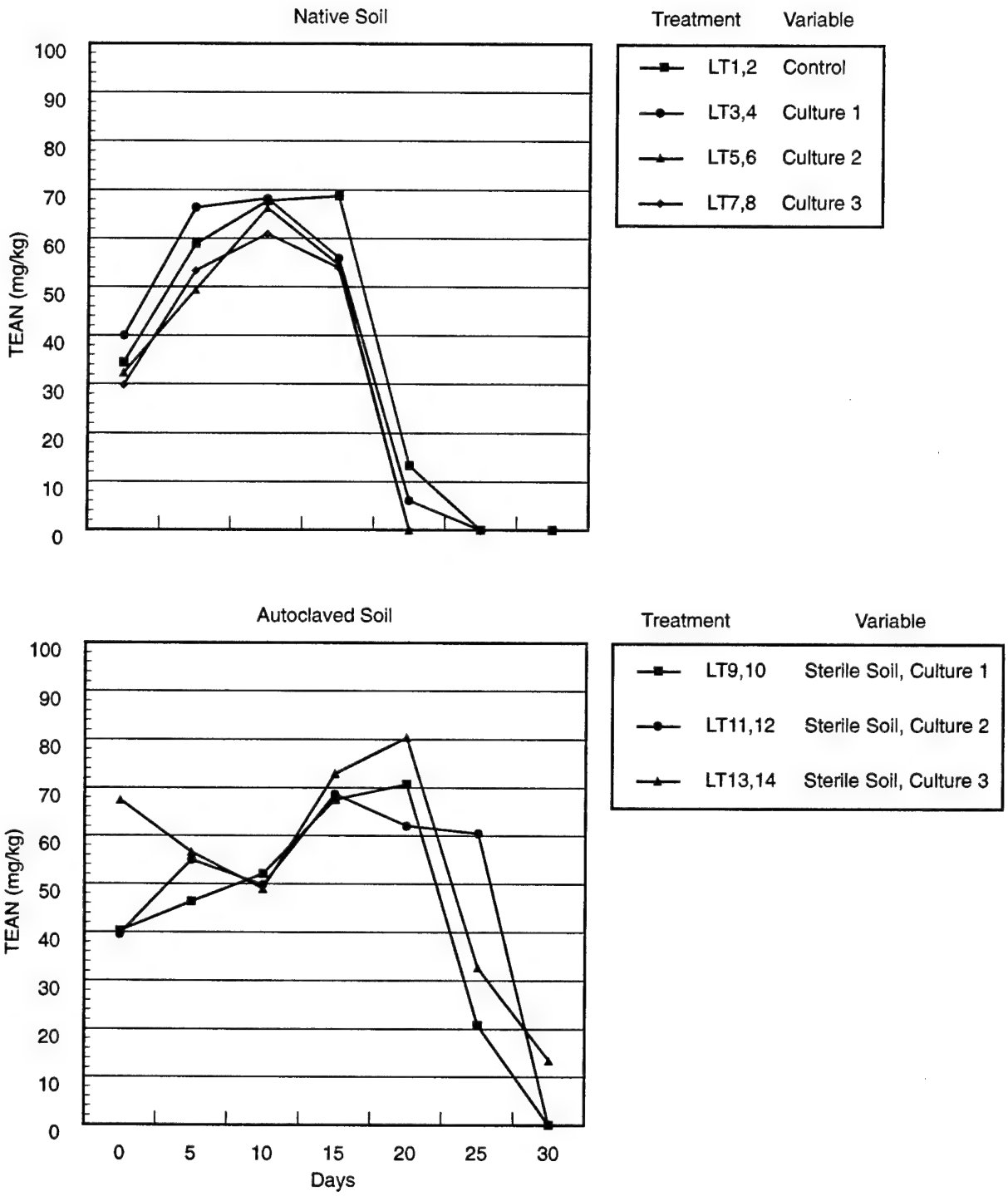


Figure 5.
TEAN Degradation Observed During Soil Matrix Test.

(soil treatments as defined in Table 4)

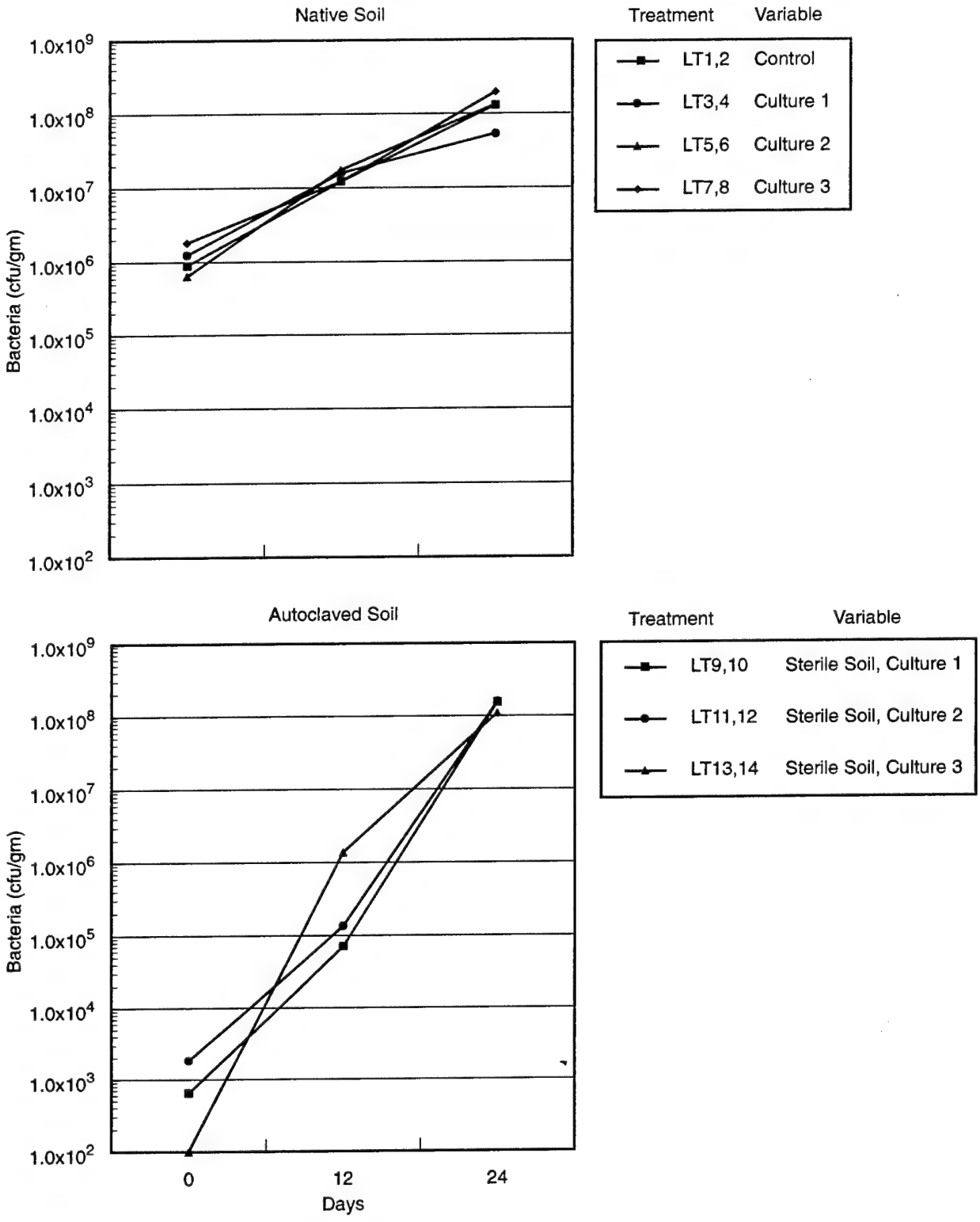


Figure 6.
Growth of Microbial Populations During Soil Matrix Test.

(soil treatments as defined in Table 4)

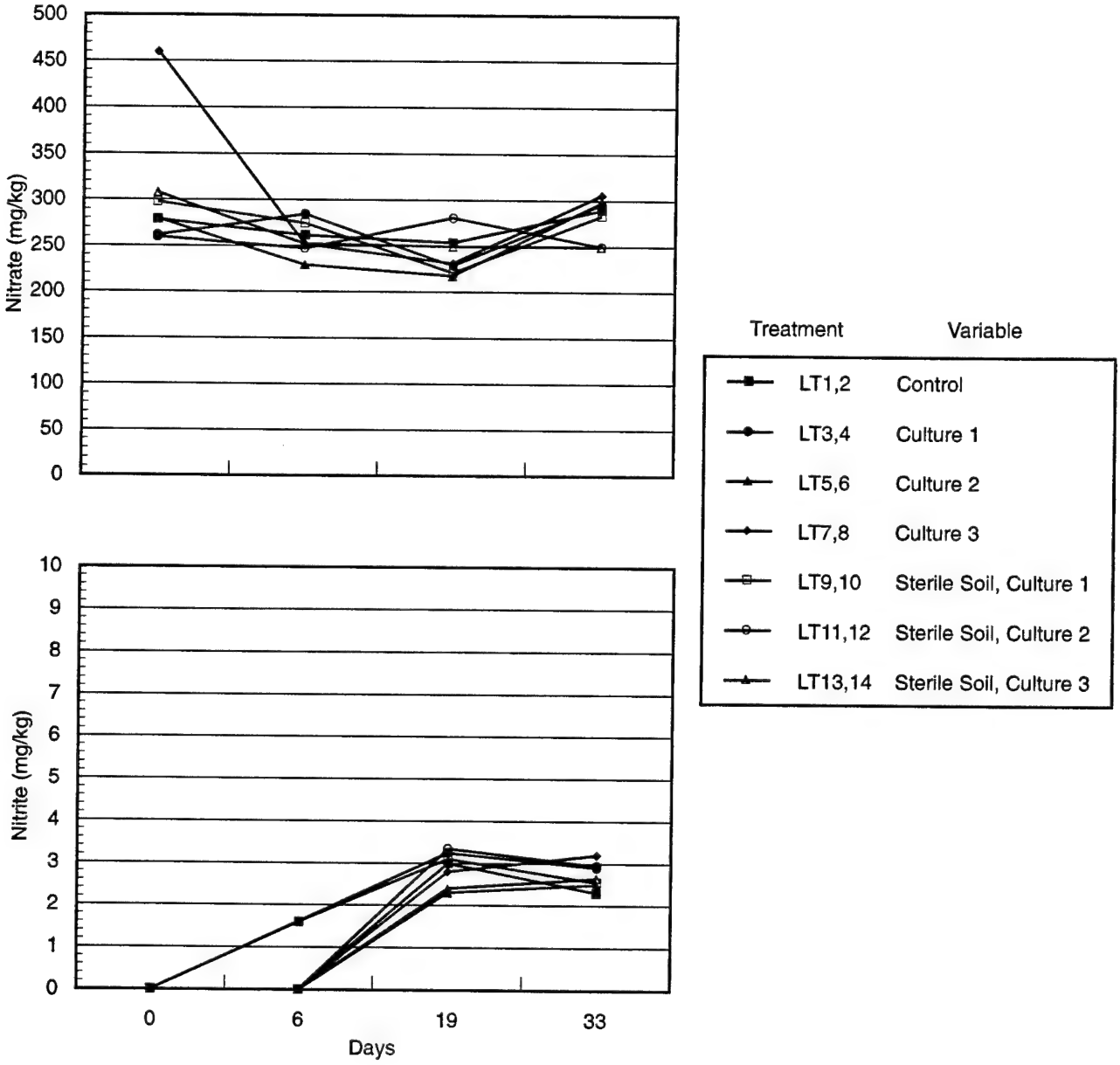


Figure 7.
Nitrate and Nitrite Concentrations During Soil Matrix Tests.

(soil treatments as defined in Table 4)

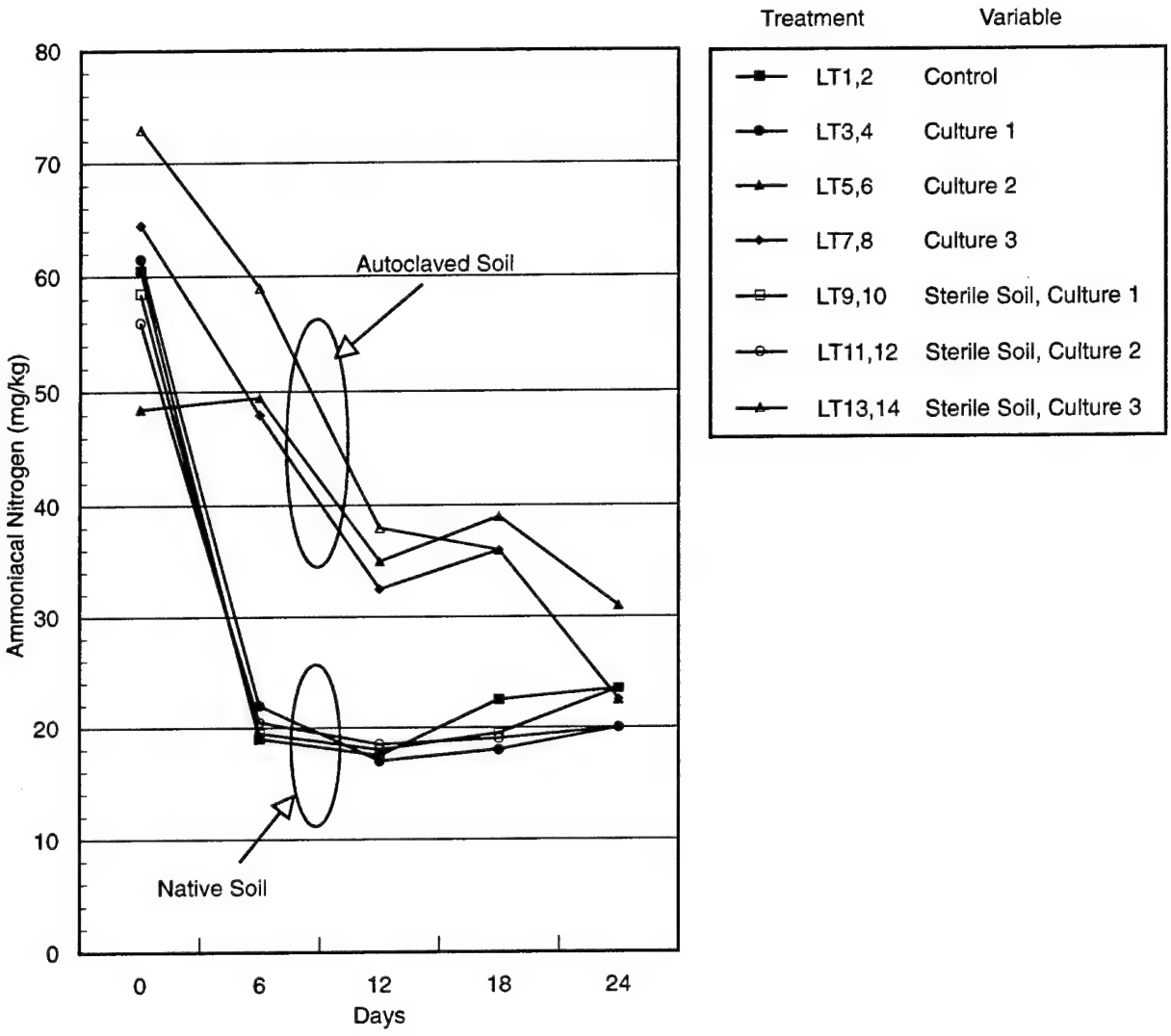


Figure 8.
Ammonium Utilization During Soil Matrix Test.

(soil treatments as defined in Table 4)

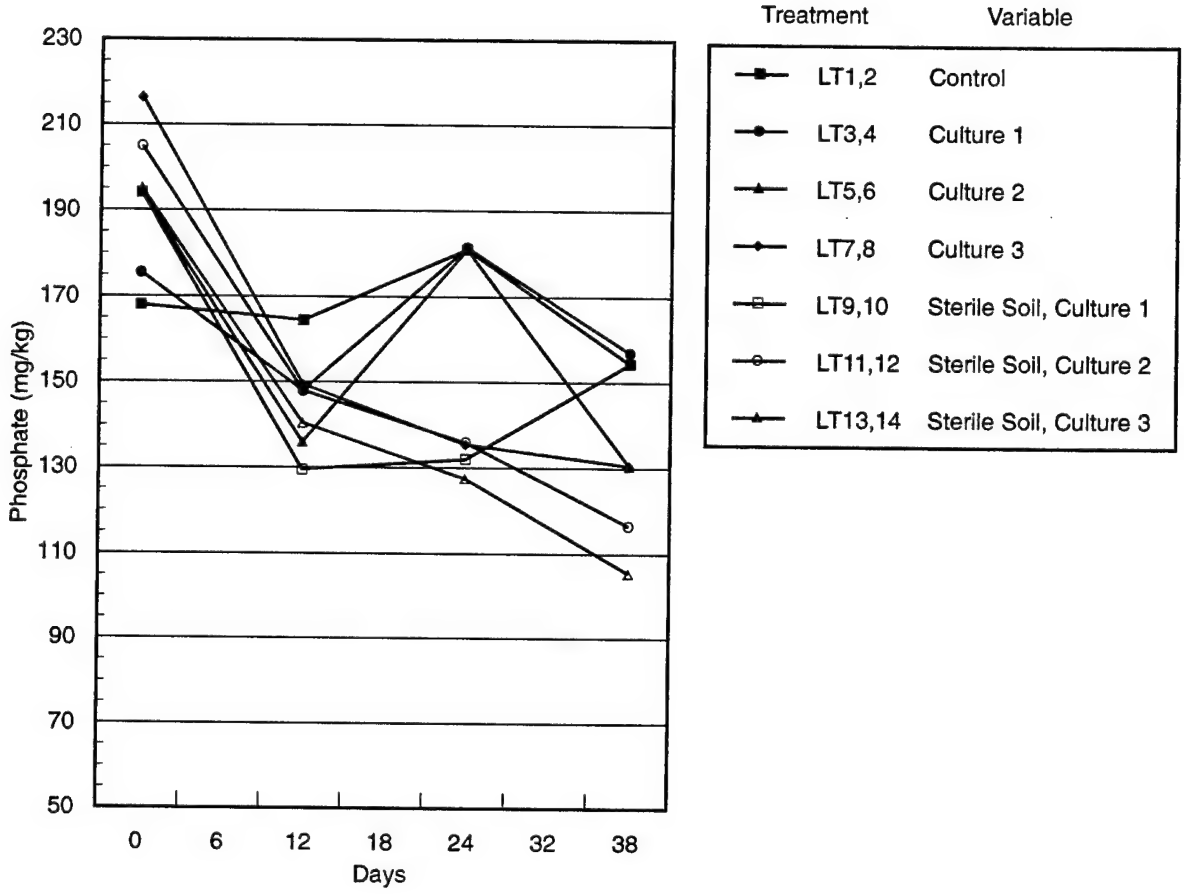


Figure 9.
Phosphate Utilization During Soil Matrix Test.

(soil treatments as defined in Table 4)

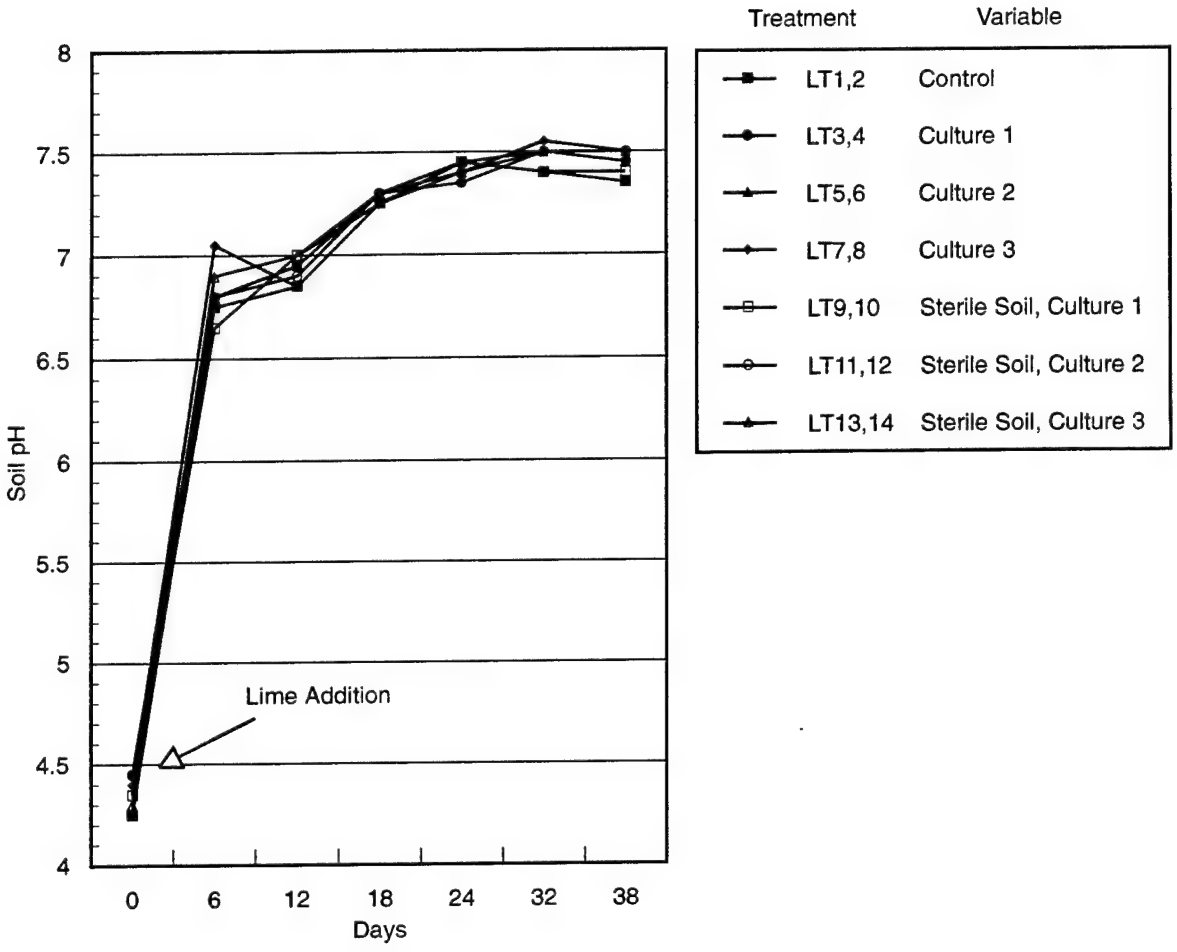
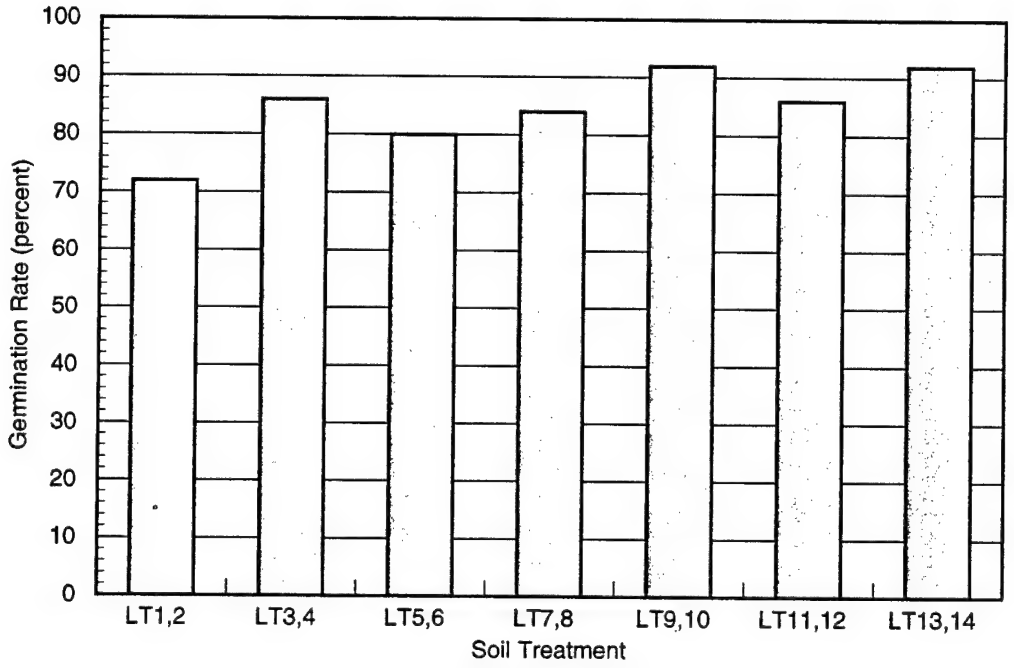


Figure 10.
Soil pH During Soil Matrix Test.

(soil treatments as defined in Table 4)



Treatment	Variable
LT1,2	Control
LT3,4	Culture 1
LT5,6	Culture 2
LT7,8	Culture 3
LT9,10	Sterile Soil, Culture 1
LT11,12	Sterile Soil, Culture 2
LT13,14	Sterile Soil, Culture 3

Figure 11.
Germination of Kentucky 31 Fescue Seeds on Soil Matrix Treatments After Completion of Soil Matrix Tests.

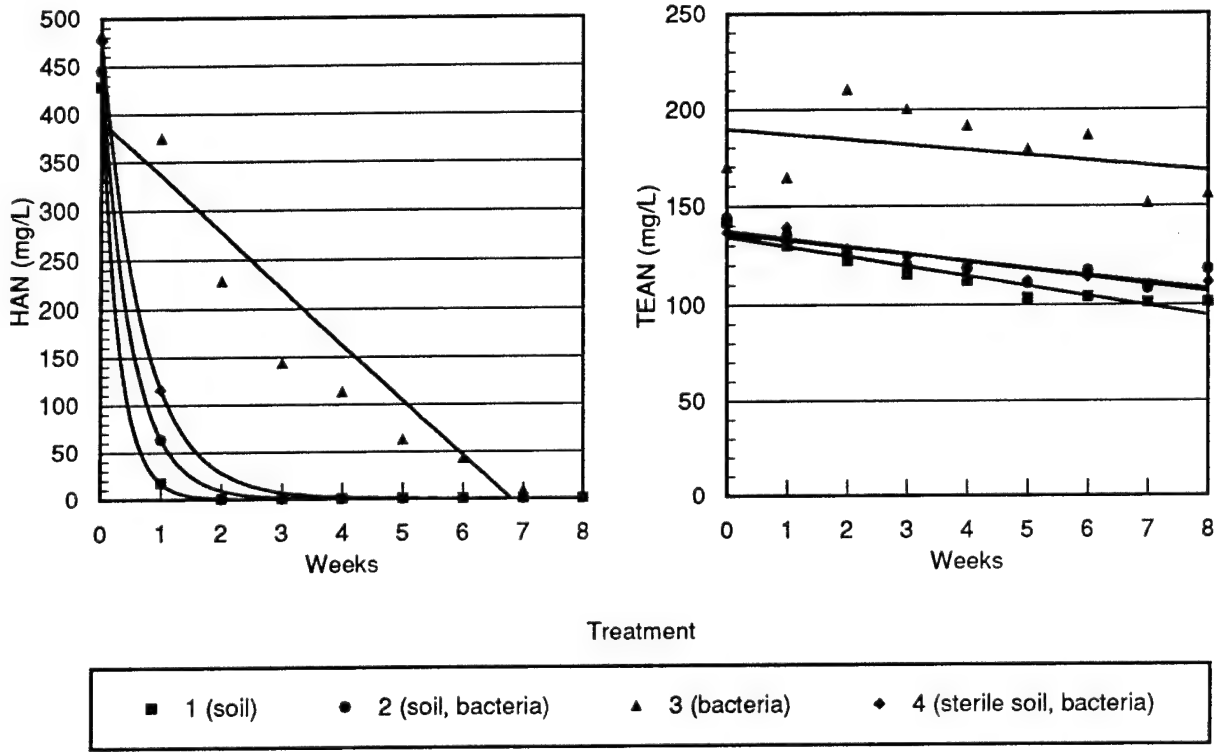


Figure 12.
HAN and TEAN Degradation During Water Matrix Tests.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

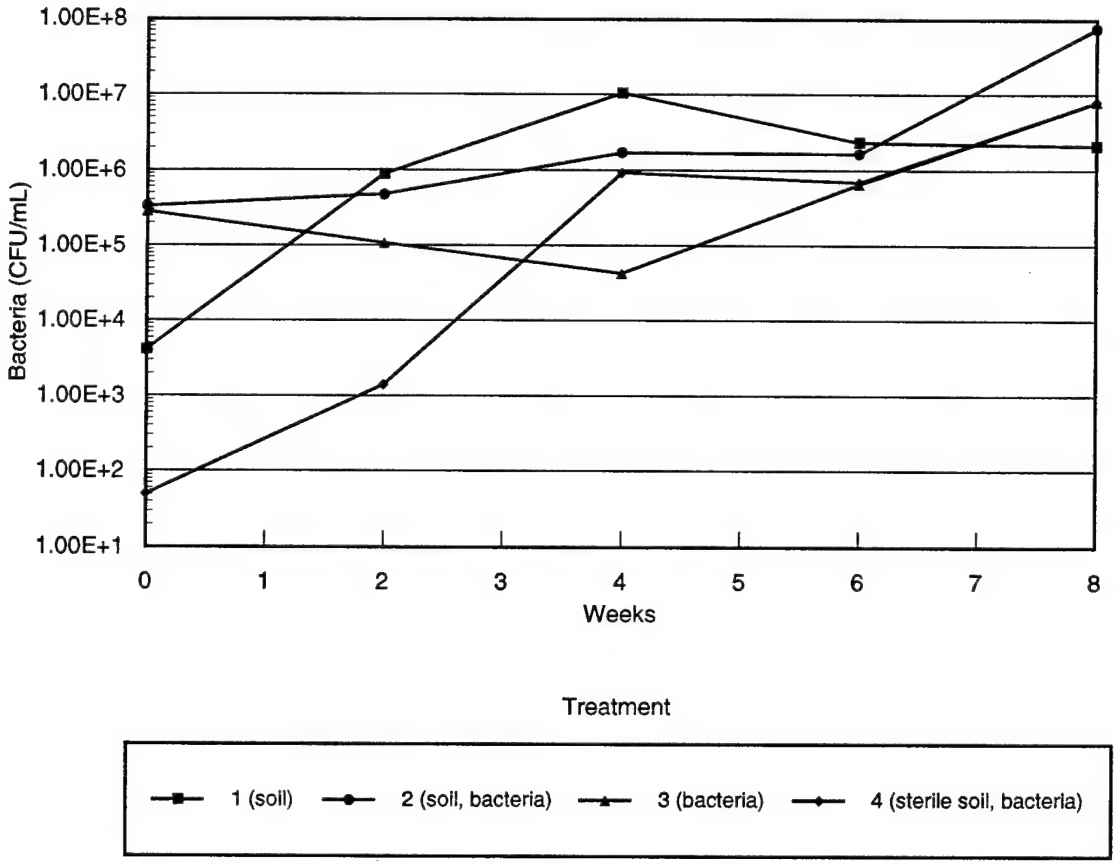


Figure 13.
Bacterial Density During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

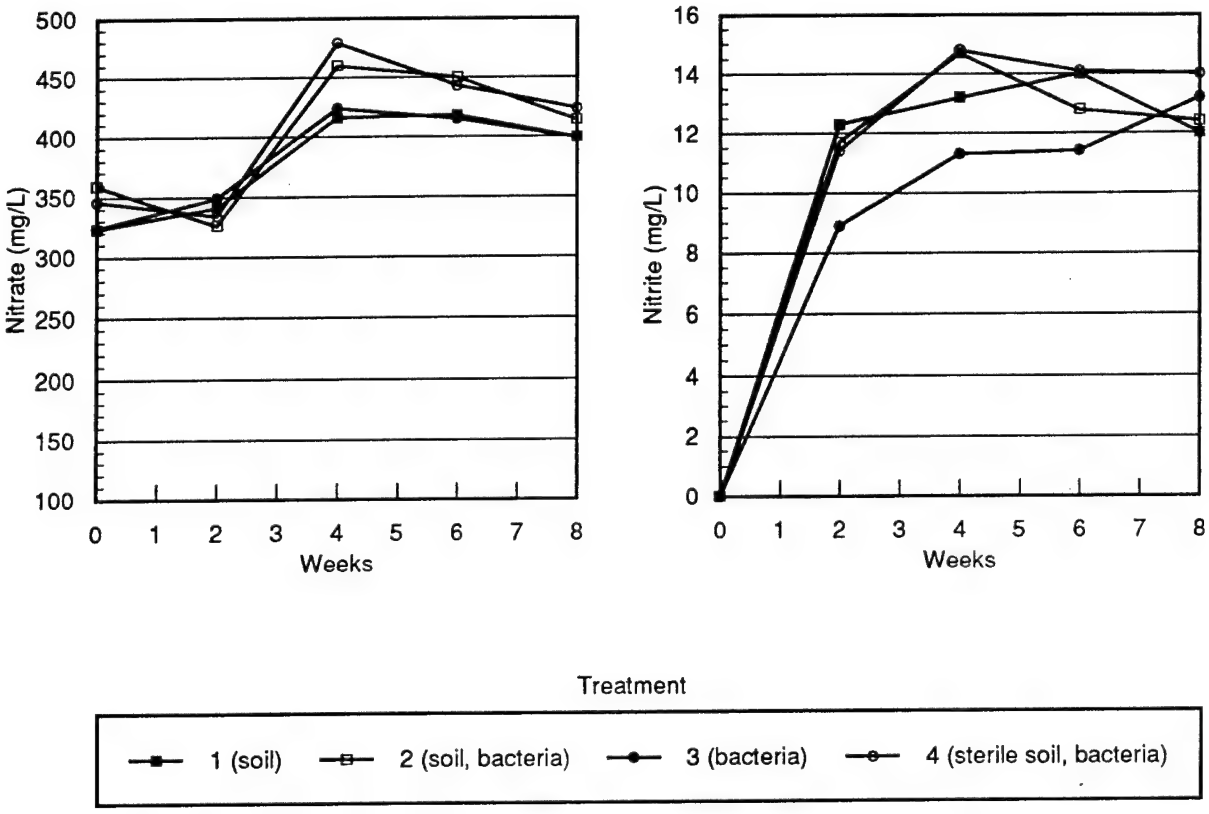


Figure 14.
Nitrate and Nitrite Concentrations During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

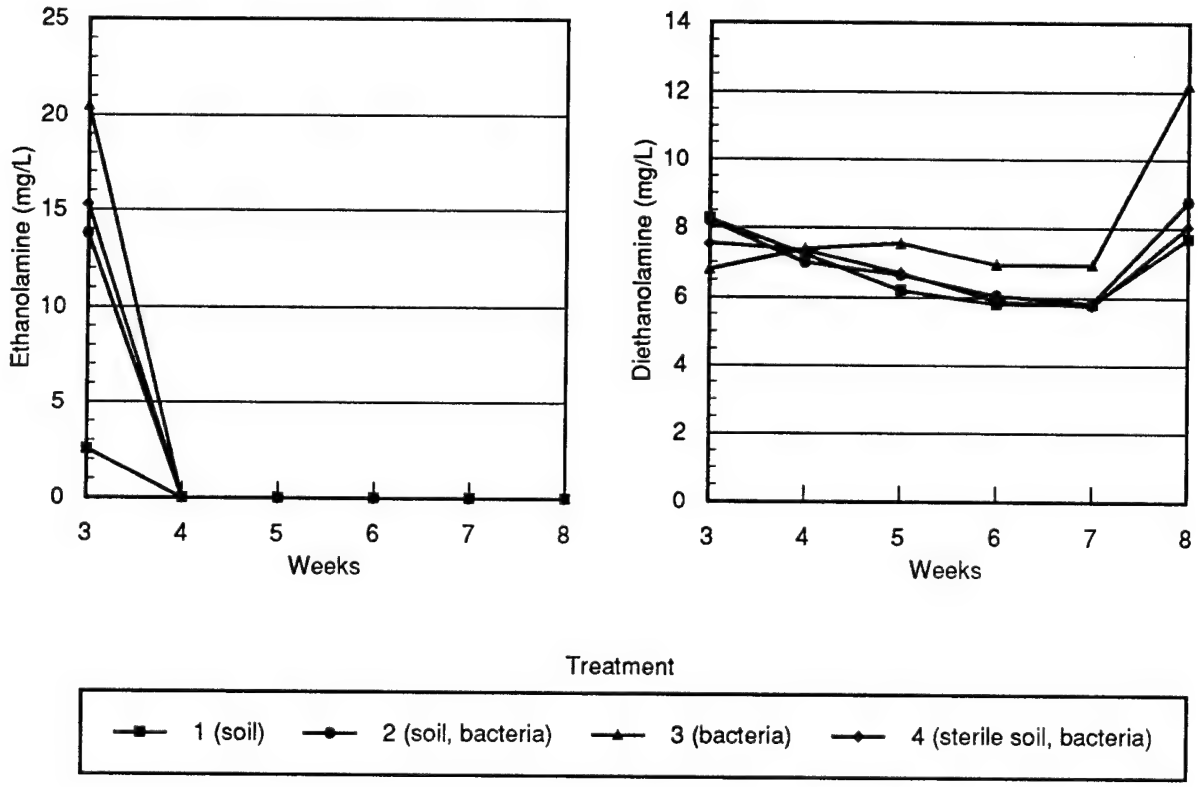


Figure 15.
Ethanolamine and Diethanolamine Concentrations During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

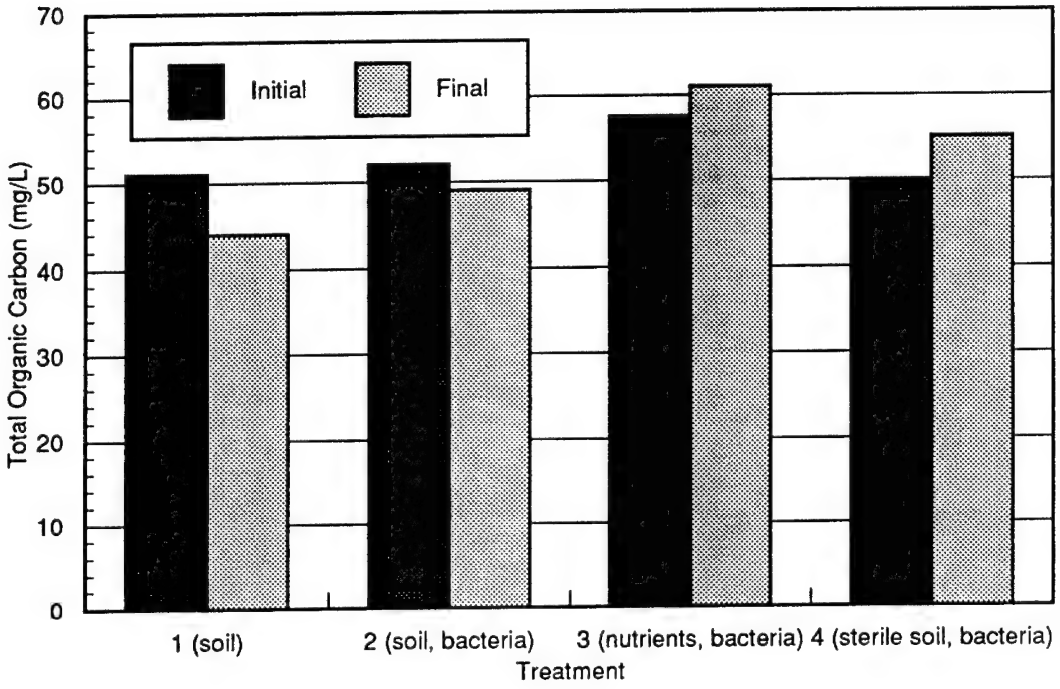


Figure 16.
Concentration of Total Organic Carbon During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

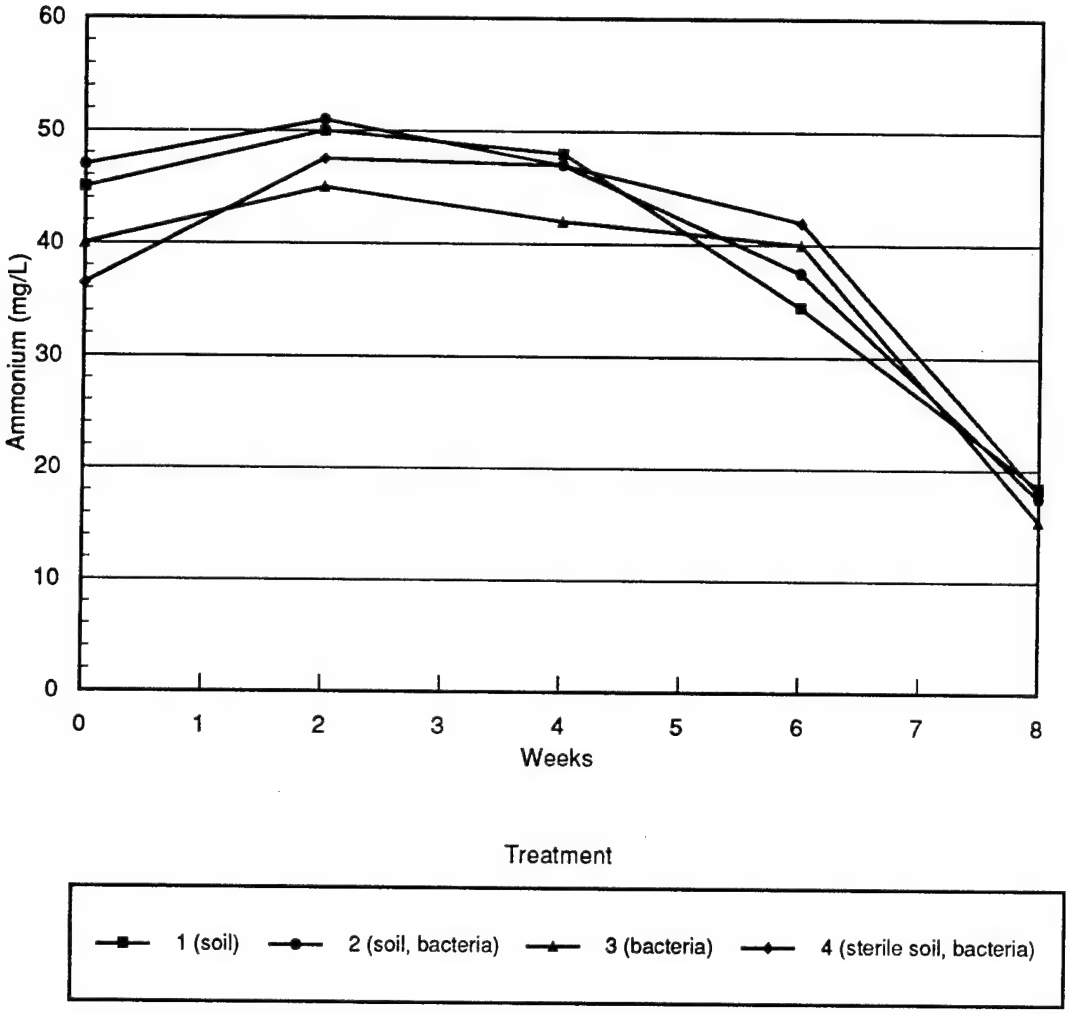


Figure 17.
Ammonium Utilization During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

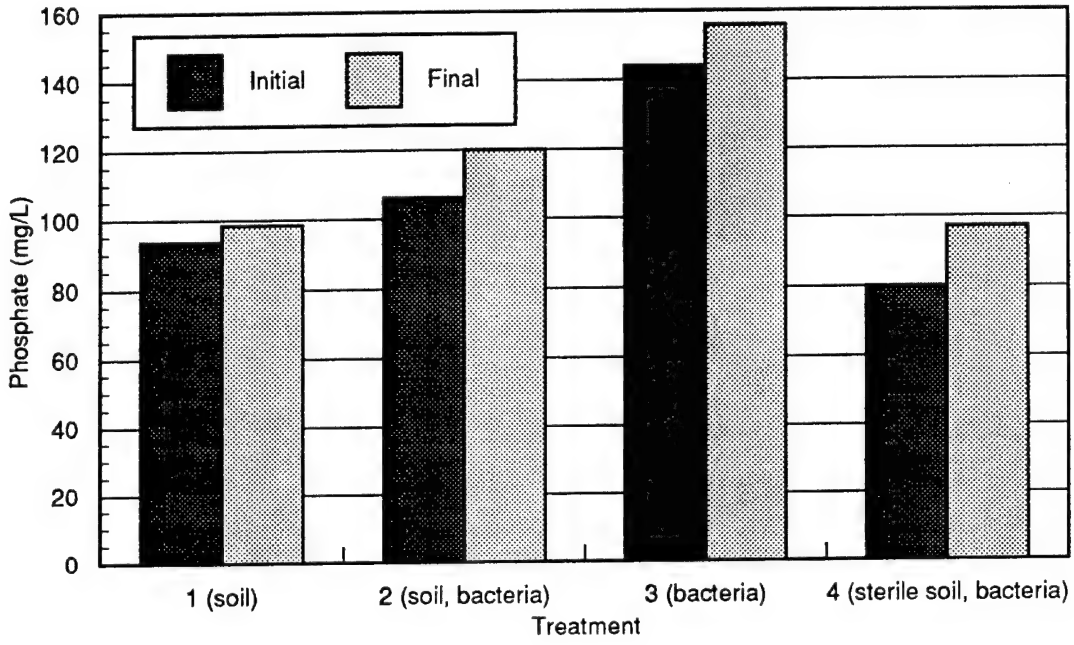


Figure 18.
Phosphate Utilization During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

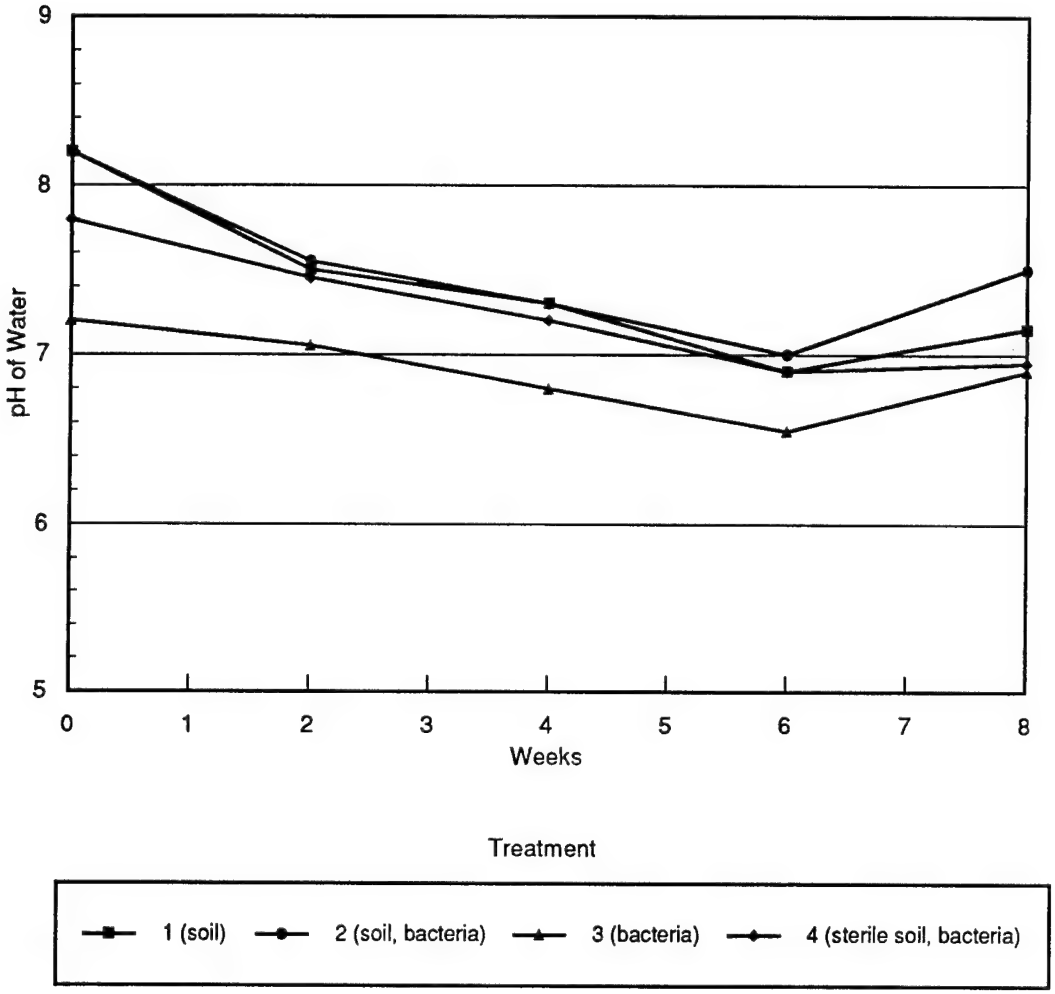


Figure 19.
pH During Water Matrix Test.

(water matrix treatments as defined in Table 6; all treatments include water, nutrients, pH adjustment, and LGP plus variables as indicated in the legend above)

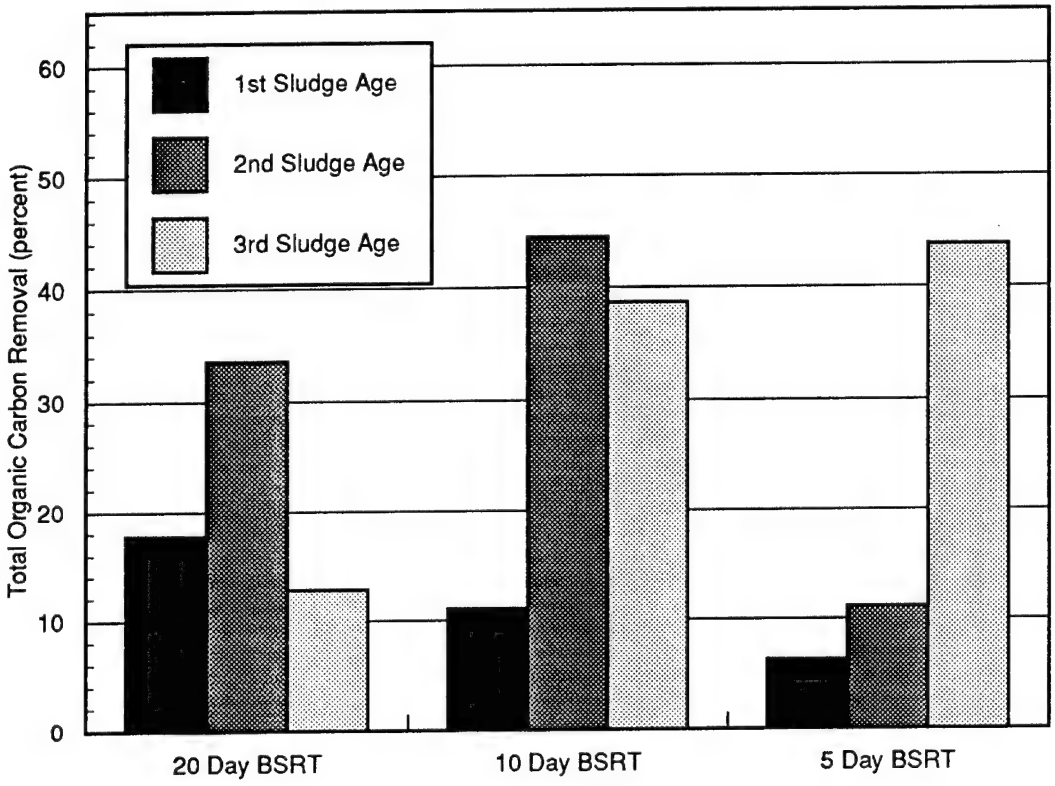


Figure 20.
Total Organic Carbon Removal During SBR Test.

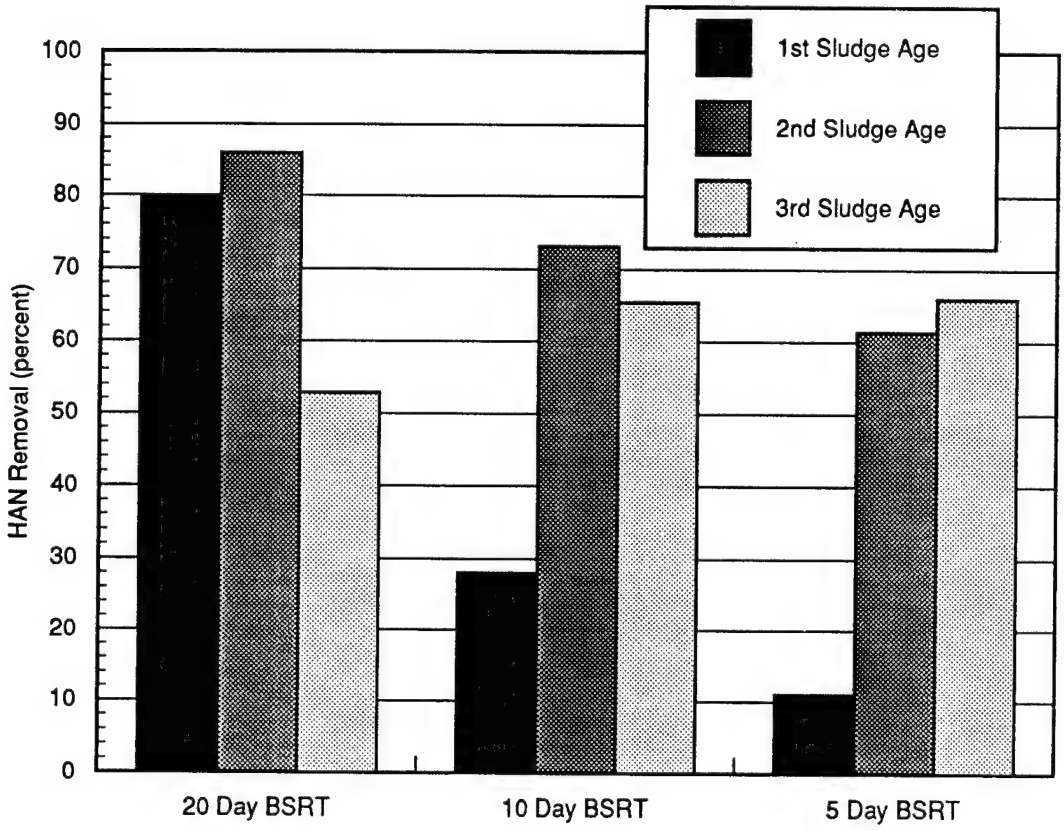


Figure 21.
HAN Removal During SBR Test.

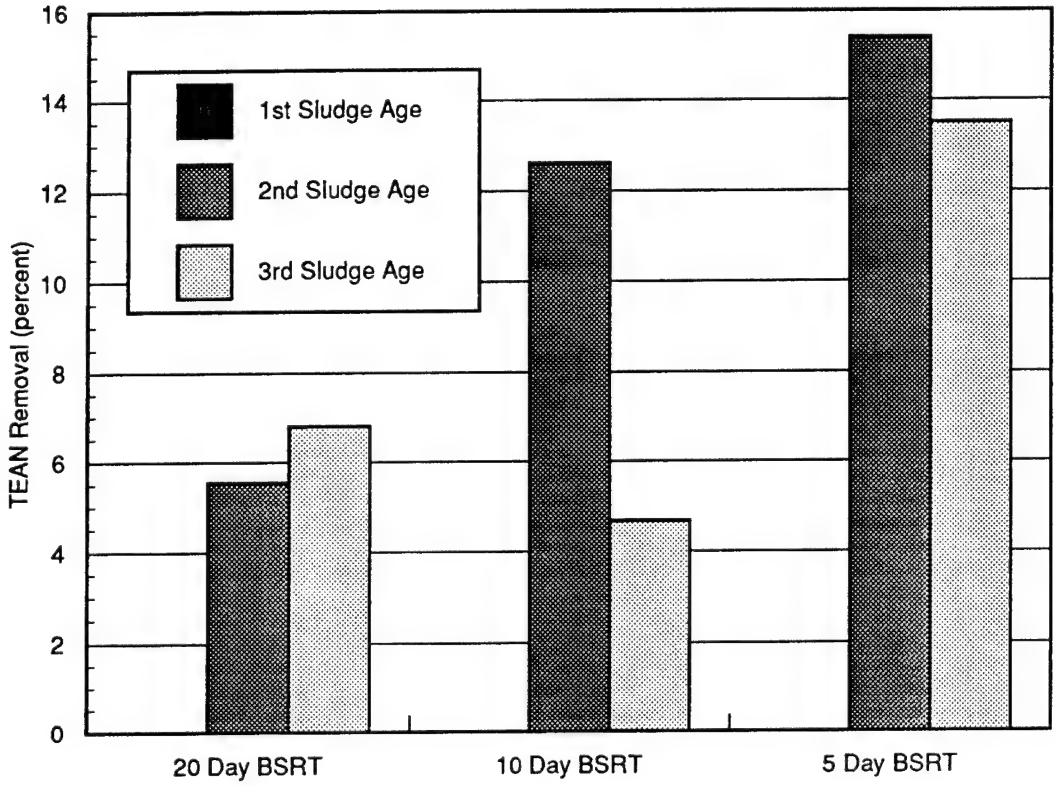


Figure 22.
TEAN Removal During SBR Test.

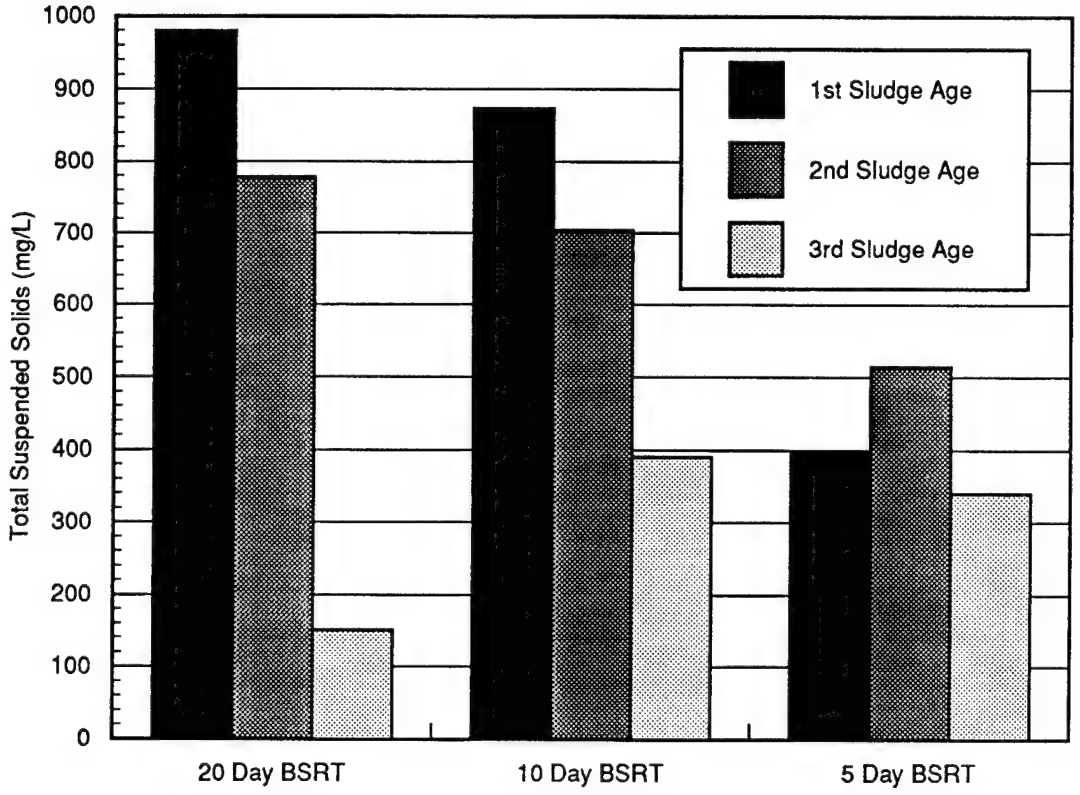


Figure 23.
Total Suspended Solids During SBR Test.

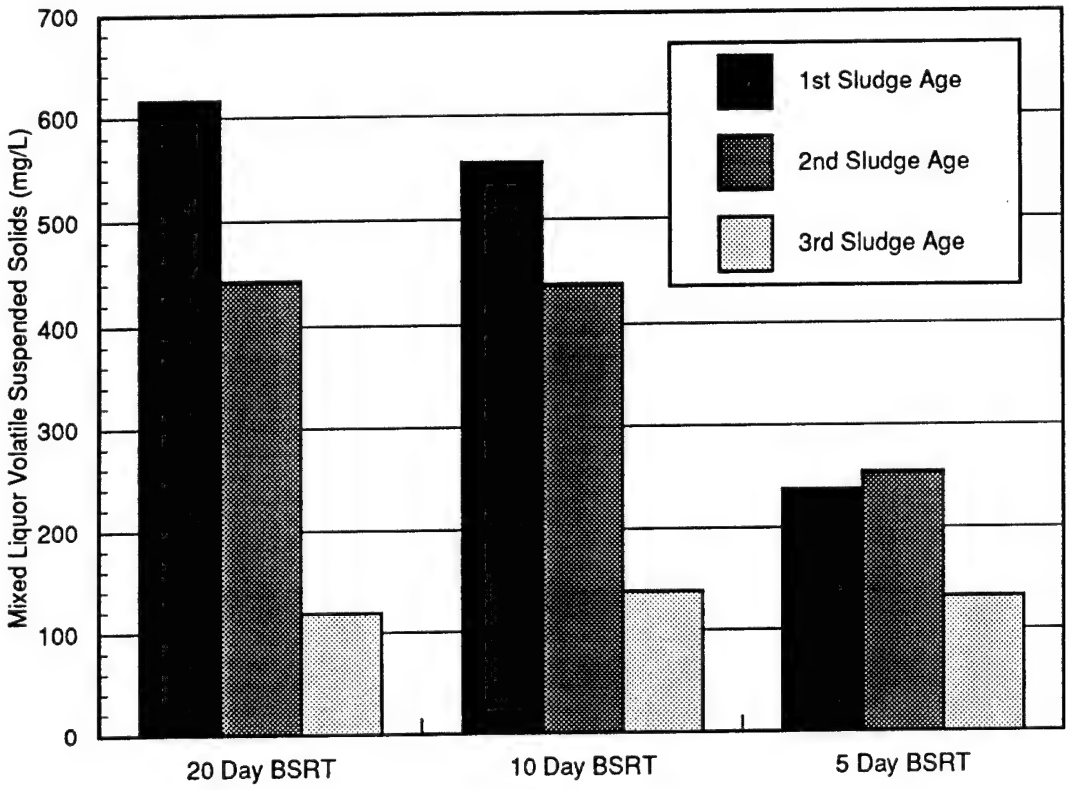


Figure 24.
Mixed Liquor Volatile Suspended Solids During SBR Test.

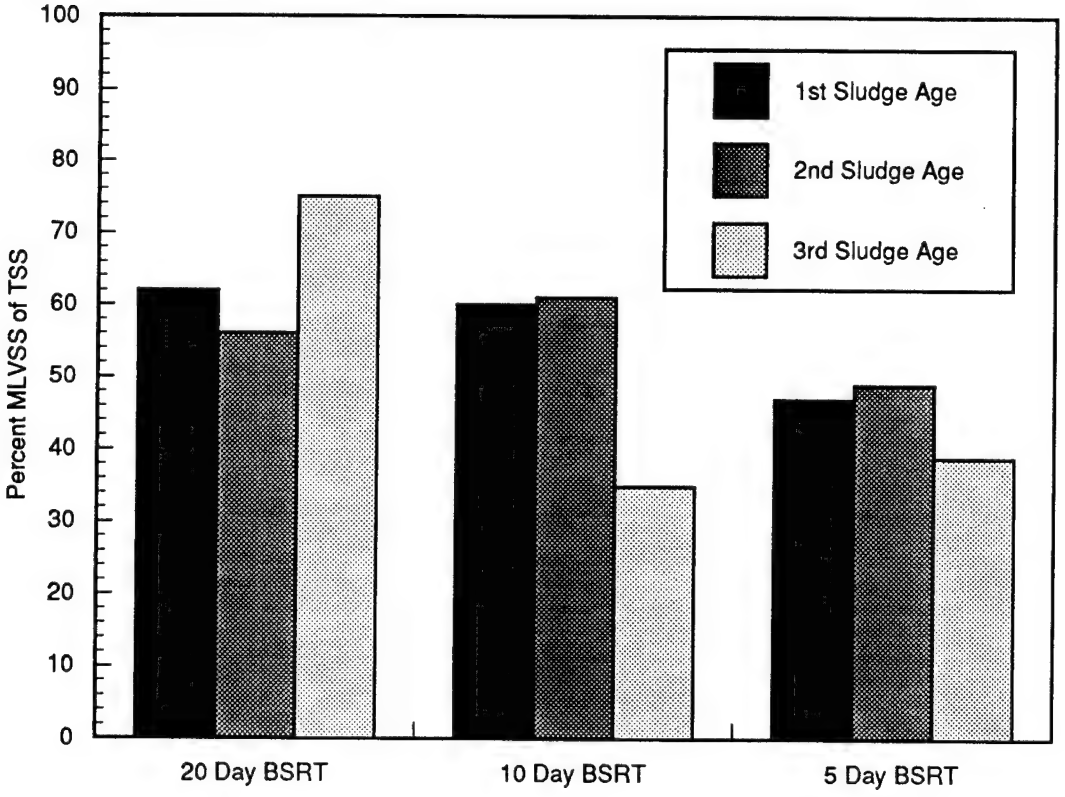


Figure 25.
MLVSS Contribution to TSS During SBR Test.

Appendix A

Assessment of Validity of Ion Chromatographic Method for Determining HAN and TEAN in Soil, Water, and Nutrient Broth

Assessment of Validity of Ion Chromatographic Method for Determining HAN and TEAN in Soil, Water, and Nutrient Broth

Prepared by

IT Corporation
Biotechnology Applications Center
Knoxville, Tennessee

Contract No. DACA31-91-D-0074
Task Order No. 2
IT Project No. 322240

AEC Project Officer
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U. S. Army Environmental Center
Aberdeen Proving Ground, Maryland

December 22, 1993

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1.0 Scope and Application

IT Corporation (IT) is currently evaluating microbial degradation of liquid gun propellant (LGP, specifically LGP 1846) under USATHAMA Contract No. DACA31-91-0074. During this evaluation LGP has been found to inhibit microbial growth at lower concentrations than anticipated. As a result, the concentrations of LGP 1846 (which consists of 19% triethanolammonium nitrate [TEAN], 61% hydroxylammonium nitrate [HAN] and 20% water) being used in the evaluation also are lower than anticipated.

Very sensitive methods exist for the detection of HAN. By oxidizing HAN to nitrous oxide, HAN can be detected at low part per billion concentrations (support documents are cited in IT's report entitled "Documentation of Existing Methods for Quantitation of TEAN"). Therefore, HAN can be easily and reproducibly detected at concentrations adequate to satisfy the analytical requirements of this investigation.

The published analytical methods for quantification of TEAN do not meet performance requirements necessitated by low concentrations LGP used in the biodegradation studies. (For a review of pertinient literature see IT's report "Documentation of Existing Methods for Quantitation of TEAN".) Accurate quantitation of TEAN was required to assess the degree of microbial degradation of LGP. Therefore, development of an acceptable analytical method was required prior to continuation of the study. An analytical method that can quantitate levels of TEAN to 1 ppm without problems caused by sample matrix components was required for completion of this study. Such a method will also be required in future evaluations of LGP spills and for evaluation or development of associated clean-up technologies.

The analysis of TEAN as a pure compound or mixed with HAN and water to form liquid gun propellant is a relatively simple process. Documented procedures include methods developed for gas chromatography, high performance liquid chromatography, thin layer chromatography, infrared spectroscopy, and potentiometric titration. Each of these methods performs well with clean samples containing known constituents and high concentrations of TEAN. General limitations for analyzing low concentrations of TEAN result from the fact that TEAN is water

soluble and very difficult to extract and concentrate. This is particularly true when the sample contains other water soluble constituents that interfere with TEAN analysis, as is the case with most environmental samples.

1.1 Potentially Applicable Analytical Techniques

Several methods have been documented that describe the analysis of TEAN. In general they are best suited for the analysis of pure liquid propellant LGP or concentrated solutions of TEAN. Little attention has been given to defining interferences, developing reliable extraction and recovery techniques for preparing environmental samples for TEAN analysis, and developing methods capable of quantifying low concentrations of TEAN. Robust methods of sample preparation and analysis are required to determine the persistence of TEAN in the environment and to provide an analytical foundation for environmental monitoring and remediation in the event of LGP releases. The chemical characteristics of TEAN suggest that the best method will be one which will detect TEAN in dilute aqueous samples.

A method under development at the U. S. Army Corps of Engineers Waterways Experiment Station (WES) in Vicksburg, Mississippi has been made available to IT Corp. The method was adequately advanced to warrant testing for this project. The WES Method employed ion chromatography with pulsed electrochemical detection.

This report describes method validation activities employed to evaluate the use of the WES HPLC-based methodology for TEAN and HAN analysis in environmental samples.

As described by IT in the document titled "Documentation of Existing Methods for Quantitation of TEAN," HPLC, specifically the WES Method, offers the best combination of characteristics for addressing the problems associated with TEAN analysis. Therefore, methods development activities were directed at validating the WES procedure.

HPLC is amenable to direct analysis of aqueous samples. The water solubility of TEAN makes fractionation of dissolved TEAN into an organic solvent impossible; therefore, the ability to directly analyze an aqueous sample is advantageous for TEAN analysis. Although HPLC is a routine laboratory technique, requisite equipment and operating conditions will make the method difficult to implement in contract analytical laboratories.

1.2 Objectives

This effort validated and documented the performance of the WES Method for accurate and precise quantification of dilute concentrations of TEAN and HAN in groundwater, seawater, three types of soil, and nutrient broth. IT understands that further development of the method as an EPA standard method will be the responsibility of WES. IT will share results with WES since this work may be beneficial to their efforts in documenting the reproducibility of their method in an independent laboratory.

2.0 Summary of WES Method

The WES method was developed to specifically achieve the detection of low concentrations of TEAN and HAN as LGP in environmental samples containing these compounds. The method, based on ion chromatography, employs a cation exchange column that simultaneously separates mono- and divalent cations and low molecular weight amines and alkanolamines. Following elution from the column, the sample is mixed with sodium hydroxide via a post-column reaction system before passing through the detector. A pulsed electrochemical detector with a gold working electrode and a sodium hydroxide saturated sodium chloride/silver chloride reference electrode was used to detect HAN and TEAN.

Detector linearity was documented over a range of 3 to 30 mg/L for HAN and 1 to 10 mg/L for TEAN. A minimum detection limit for HAN was calculated to be 20 µg/L using three times the background noise level. The minimum detection limit for TEAN was calculated to be 220 µg/L. The retention time for HAN was 3.33 minutes and 6.42 minutes for TEAN. Variability of triplicate analysis of the same sample was reported to be less than 3 percent for HAN and less than 7 percent for TEAN (personal communication, Ann Strong and Don Rathburn, WES, 1993). These results were determined using TEAN and HAN solutions prepared in deionized water.

WES also reported that no Standard Analytical Reference Material was available for HAN and TEAN (personal communication, Don Rathburn, WES, 1993). Therefore, calibration standards were prepared from a previously analyzed sample of LPXM46 (their working LGP formulation).

3.0 *Equipment and Materials*

The equipment and materials used to execute the ion chromatography procedure are described. The major pieces of hardware such as the liquid chromatograph, the auto sampler, and the detector were not manufactured by the same company as the equipment used by WES. However, chromatography columns and chromatography solutions were exactly as specified by WES. Operating conditions were established based on WES guidance. Soil extraction procedures were developed by IT.

3.1 High Performance Liquid Chromatography

A Dionex Liquid Chromatograph Model DX-300 equipped with a Spectra-Physics SP8880 autosampler was used throughout the method validation program. A 10 µL sample loop was found to provide adequate sensitivity and the best resolution of HAN, TEAN, ethanolamine, and diethanolamine peaks. Integration and analysis of the chromatograms was accomplished with Dionex AI-450 Chromatography computer software. Lack-of-fit and regression analyses of calibration data were performed using Microsoft's Excel spreadsheet.

3.2 Ion Chromatography Column

A Waters IC-PAK Cation Exchange Column Model No. 36570 was used to resolve HAN and TEAN.

3.3 Post Column Reactor

A Dionex Post-column Pneumatic Controller delivered 0.3 M sodium hydroxide to the eluent stream. The mixing unit and reaction coil ensured complete mixing and adequate contact time of the sample with sodium hydroxide before detection of HAN and TEAN.

3.4 Pulsed Electrochemical Detector

A Dionex Pulsed Amperometric Detector (PAD) with a gold working electrode and a silver/silver chloride reference electrode were used to detect HAN and TEAN. Settings for the detector, as specified by WES, are listed below:

E1, 100 mV
T1, 20 cycles 0.333 seconds

E2, 880 mV
T2, 20 cycles 0.333 seconds
E3, -520 mV
T3, 10 cycles 0.333 seconds
Total pulse time, 0.999 seconds
I Range, 0-10 microamperes

E1, E2, and E3 were the voltages applied to the eluant as it passes through the detector electrode cell. E1 was the voltage controlling the reduction of the analyte. E2 and E3 were voltages applied to condition the electrodes. T1, T2, and T3 were the time intervals during which each voltage was applied to the electrode cell. I was the electrical current range.

The following settings were employed during method validation:

E1, 100 mv to 150 mv
T1, range 2, 300 seconds
E2, 880 mv to 930 mv
T2, range 2, 300 seconds
E3, -520 mv to -470 mv
T3, range 2, 300 seconds

E1, E2, and E3 were adjusted up or down as needed to increase sensitivity to TEAN.

3.5 Sonication

Soil samples were extracted using a Tekmar Sonic Disruptor Model TM375 with disruptor probe Model CV17. Two gram soil samples were slurried with solvent in a glass vial prior to extraction. Sonication parameters were 200 seconds at a 20 percent pulsed duty cycle, output 5, with the sample held on ice.

4.0 Reagents

The eluant for the chromatographic separation of triethanolamine and hydroxylamine consisted of five percent methanol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 3

mM of ultra-pure nitric acid. Sodium hydroxide (0.3 M) prepared in carbon dioxide-free water was used as a post column eluant to ionize triethanolamine and hydroxylammonia.

The eluant flow rate was 0.9 mL per minute through the column with a 0.2 mL to 0.25 ml per minute flow of post column eluant added down stream of the column. The flow rate of post column eluent was adjusted to enhance the ionization of TEAN, thus improving the detectability of TEAN.

5.0 Instrument Calibration

A variety of calibration standards were used to evaluate the response of the chromatographic system. Table 1 outlines the single compound solutions and mixtures used to generate calibration curves and to determine linearity and functional detection limits. All solutions were prepared using sterile deionized water.

Regression analysis and lack-of-fit testing using the F-test at the 95 percent confidence level indicated a highly significant linear relationship between concentration and the response generated by the detector. The data, regression analysis, and statistical results from each calibration series are provided in Appendix A.

A six or seven point calibration series was run with every batch of samples. Linear regressions and statistical analysis were performed and the regression equations were used to determine HAN and TEAN concentrations in each test sample.

6.0 Quality Assurance and Quality Control

6.1 Calibration

Calibrations were performed with five or six standards prepared in deionized water. Table 1 shows the calibration sets used to verify method performance and to determine detection limits, detection range, and linearity. Table 2 indicates the calibration sets used during the analysis of LGP in environmental matrices (discussed in Section 7). Instrument calibration

was verified after every 10 samples by running a single calibration standard. Intra- and inter-day variability of the standards was also evaluated. Linear regression was performed on calibration data using a Single Classification Model I Analysis of Variance and the F-test to determine the amount of linear variation in the measurement accounted for by variation in concentration. This approach is a "lack-of-fit" test for determining the linearity of a regression model as per the U.S. Army Toxic and Hazardous Materials Agency Quality Assurance Program. A significant regression at the 95 percent confidence level was considered acceptable. If the regression model was not significant at the 95 percent level, new standards were generated and/or instrument diagnostics were conducted. After every tenth sample a calibration standard was run to determine the variability of the method.

During initial testing of the method and verification of the detection of HAN and TEAN, "lack-of-fit" tests were conducted on calibration sets containing TEAN, HAN, LGP, and a mixture of HAN, TEAN, ethanolamine, and diethanolamine. The results are provided in Appendix A. Because the "lack-of-fit" test always indicated a highly significant (much greater than 95 percent) linear relationship between detector response and concentration for each test compound, routine calibration during subsequent tests was automated using the data analysis software associated with the HPLC. Correlation coefficients greater than 0.98 were calculated for all sets of calibration data.

6.2 Method Blanks

Method blanks were used to document contamination resulting from laboratory processing. Method blanks contained all reagents in the same volumes and proportions used in sample processing. Method blanks were handled in the same manner as actual samples except they were not exposed to the test material. Triplicate method blanks were run with each set of samples. Triplicate unspiked groundwater and seawater samples were incubated with the aqueous samples in section 6., the blanks were analyzed along with the aqueous samples. During soil extractions, the extraction solvents, deionized (DI) water, 3M potassium chloride, and methanol, were sonicated and analyzed in triplicate along with the samples. Similarly, triplicate samples of untreated soil were analyzed. In cases where a background peak eluted at the same time as a test compound, background was manually subtracted from the target compound peak. The magnitude of background peaks is indicated as a footnote on appropriate data tables.

6.3 Matrix Spikes

Method validation activities require samples that are prepared from clean matrices spiked with TEAN. Therefore, additional matrix spikes were not required. However, matrix spikes will be employed during biodegradation studies. This will be done by spiking samples with a known amount of LGP. The spiked sample will be compared to the unspiked sample to determine recovery efficiencies. Matrix spikes will be analyzed at a 10 percent frequency or at least one per day.

6.4 Precision

Method precision describes how close multiple measurements of the same sample are to each other. Precision was measured by examining the results of multiple analyses of samples containing a known amount of LGP components. Precision was calculated in two slightly different ways. In the first approach, precision was calculated by determining the percent standard deviation of the observed mean concentration. The second approach determined precision by finding the percent standard deviation of the mean recovery. Results are comparable using either approach.

Several tables of method precision data are presented because various factors influence the precision of the method. Each observation is discussed in order to describe the potential sources of error that may occur during analysis.

Quality assurance (QA) samples were run with each set of analyses. These samples were single calibration standards prepared in DI water. A QA sample was analyzed after every ninth sample (10 percent frequency). The results of QA samples run during the analysis of aqueous samples are shown in Table 3. Tables 4 and 5 show the method precision calculated during soil extractant testing and immediate aqueous extraction of soil spiked with LGP, respectively. Table 6 shows precision calculations for aqueous extracts of soil spiked with LGP and allowed to incubate at 4°C for six days. Method precision was calculated as the percent standard deviation of the mean recovery in each table.

The results from Tables 3, 4, and 5 indicate a method precision of 6 to 11 percent for TEAN and 6 to 10 percent for HAN. These results indicate that the detection of HAN and TEAN is reproducible and consistent during the analysis of a sample set containing at least 30 samples. The results from Table 6 indicate otherwise. The precision of TEAN measurements was +/-

41 percent. The precision of HAN measurements was +/- 13 percent. The obvious deviation of these results from those in Tables 3, 4, and 5 suggested an interference related to the sample type rather than the method. The cause for the aberrant TEAN precision has not been identified; however, this observation indicates the potential difficulties associated with the analysis of soil. Precise analysis of soil extracts may require more frequent instrument calibration or a conditioning step in the analytical program to restore the column or detector to steady state conditions.

Tables 7, 8, and 9 indicate the precision of the method during analysis of aqueous samples calculated as the percent standard deviation of the mean concentration. Table 7 shows the overall method precision for combined groundwater and seawater samples. Tables 8 and 9 show separate precision calculations for groundwater and seawater analyses. The precision of measurements of three different concentrations of TEAN and HAN is given in each table. The relatively poor precision of TEAN analysis at low concentrations shown in Table 7 reflects the poor precision of TEAN measurement in seawater (Table 9). Similarly, the extremely low precision of HAN detection shown in Table 7 is caused by the inability to detect low concentrations of HAN in seawater (Table 9). Table 8 shows that analytical precision ranged from 2 to 11 percent and 3 to 8 percent for TEAN and HAN in groundwater samples, respectively.

The precision of measuring HAN and TEAN in sandy, organic, and clayey soils is shown in Tables 10, 11, and 12. Cases where low concentrations of TEAN and HAN were not detected were considered invalid measurements for computing precision. Examples of this condition were the low concentration HAN measurements in Table 10 and the low concentration TEAN measurements in Table 12. Therefore, valid measurements of TEAN analytical precision ranged from 2 to 24 percent. Valid HAN precision ranged from 2 to 22 percent.

6.5 Detection Limits

The lower practical quantitation limit for HAN and TEAN were determined by analyzing progressively lower concentrations of HAN and TEAN. Using this approach the lower quantifiable limits for HAN and TEAN in aqueous samples were determined to be 1 mg/L of each (Table 13). The upper instrumental detection limit was determined by the aqueous concentration that saturated the electrochemical detector using a 10 µL injection loop. HAN

saturated the detector at greater than 150 mg/L. TEAN saturated the detector at greater than 400 mg/L.

Measurement of LGP in soil requires the extraction of HAN and TEAN using a suitable solvent. One requirement of the extraction procedure was that sufficient solvent was added to the soil so that solvent could be recovered. For example, at least 2.5 mL of water had to be added to one gram of sandy or organic soil to insure that enough free water could be recovered to run the analysis. Because of the much greater water holding capacity of clay, 8 ml of water was added per gram of clay soil to yield enough water to conduct an analysis. The addition of solvent to soil samples resulted in an increase in the quantifiable limit due to dilution. The lower quantifiable limit for sandy and organic soil was 2.5 to 3 times greater than the lower quantifiable limit for an aqueous sample. The lower practical quantitation limit increased eight-fold over the aqueous limit for clay soil. Soil extracts could be concentrated by evaporation; however, the apparent instability of HAN and TEAN at low concentrations suggested that extract concentration was not warranted (discussed in Section 7).

6.6 Linear Range

The quantifiable range was 1 to 400 mg/L for TEAN, 1 to 150 mg/L for HAN, 0.25 to 25 mg/L for ethanolamine, and 0.5 to 75 for diethanolamine. The detector response was linear over the entire quantifiable range of each compound as demonstrated in the calibration curves shown in Appendix A. The upper instrumental detection limit for each compound was the absolute concentration that yielded a maximum response from the detector.

7.0 Sample Preparation, Extraction, and Analysis

Prior to this evaluation, the WES method had not been critically tested against samples likely to contain a variety of organic and inorganic compounds. Therefore, the method was tested using samples of clay, organic, and sandy soil,simulated seawater, groundwater, and nutrient broth each spiked with HAN, TEAN, or LGP.

7.1 Nutrient Broth

The detection of TEAN and HAN in the presence of nutrient broth was examined because LGP-tolerant microorganisms have been isolated and cultured in a nutrient broth-based

medium. Enrichment studies indicated that LGP-tolerant microorganisms require an additional carbon source to survive when LGP is present. The carbon source (nutrient broth) represents a potential interference in the detection of LGP components. Five different concentrations of LGP were added to 0.2 percent nutrient broth and analyzed using the WES method. Results are shown in Table 14. Recovery was determined by comparing the amount of HAN and TEAN as LGP added to the amount recovered. Nutrient broth contained small peaks eluting at the same time as HAN and TEAN. The areas of these peaks were equivalent to 2.99 and 2.87 mg/L HAN and TEAN respectively. The results shown in Table 14 have been corrected by subtracting the background nutrient broth peaks from the HAN and TEAN peaks.

Low recovery was observed in the 1 to 2 mg/L range for both TEAN and HAN with a range of 48 to 59%. Good recovery was observed in the 6 to 70 mg/L range for TEAN and HAN with a range of 95 to 118%.

7.2 Aqueous Sample Analysis

Groundwater and artificial seawater served as the test matrices. A drinking water well located in Knox County, Tennessee was the source of groundwater. Artificial seawater was prepared using aquarium salt (19 grams of salt per 0.5 L of deionized water). These matrices were spiked with LGP at concentrations of 2, 10, and 100 times the detection limit as identified for TEAN. The spiking concentrations of LGP were 10.5, 53, and 527 ppm, respectively. The spiked samples were analyzed after 72 hours of storage at 4°C. All samples were prepared and analyzed in triplicate. Tables 15 and 16 list the results of the analysis of aqueous samples. Percent recovered was determined by comparing the amount added to the amount recovered. Appendix B contains representative chromatograms developed during the analysis of groundwater and seawater.

The amount of TEAN measured in groundwater and seawater was consistently greater than the amount added (Tables 15 and 16). Ionic interactions between TEAN and natural groundwater constituents were speculated to cause a change in detector sensitivity to TEAN since standards prepared in deionized water appeared to be randomly distributed around the known concentration.

HAN measurements in groundwater were lower than expected, especially at lower concentrations (Table 15). A similar trend was also noted for seawater except that HAN was not detected in samples spiked with 6.4 and 32.3 mg/L HAN (Table 16). These observations suggest that HAN is less stable than TEAN, as reported in the literature, and that HAN is less stable in seawater than fresh water. It appears that there is an interaction with the cations in the seawater and HAN. Small amounts of HAN seem to decompose in seawater, while larger amounts (greater than 35) appeared to interact with the cations causing poor resolution of the HAN peak (see Appendix B seawater chromatograms).

7.3 Soil Sample Analysis

Soils are a difficult matrix to extract because of their ion chemistry, large surface area, and hydration which can shield compounds from extraction solvents. Sandy, clayey, and organic soils were examined during the development of the extraction method. Clay soil was collected from a construction site in Blount County, Tennessee. Potting soil was used as an organic soil. Sandy soil was obtained from a shallow aquifer near Chicago, Illinois.

These soil types present a broad range of characteristics to test the efficiency of extraction procedures. LGP was quantitatively added to each soil type at 2, 10, and 100 times the method quantifiable limit for TEAN in water. Two grams of soil was treated with LGP in glass vials. The spiked soils were incubated at 4°C for seven days to permit adsorption of TEAN onto the soil. Storage at 4°C is routinely used to reduce microbial activity in the soil to negligible levels. After seven days the entire vial was extracted using sonication and analyzed for TEAN and HAN.

The quantifiable limit for LGP varies with soil type because the dilution required to achieve a workable solvent volume varies with the soil type. For example, the quantifiable limit for clay was higher than for sand because more water was added to the clay to produce an extraction mixture that had recoverable water. Eight mL of water was added to each gram of clay soil, whereas only 2.5 mL was required to give excess water in sand and organic soil.

7.3.1 Solvent Selection

Communication with WES scientists indicated that procedures for extraction of LGP from soils have not been well defined. The potential for interferences in a complex extract had not been carefully examined. The extraction efficiency of LGP components from different soil types had not been previously determined. Therefore, the preparation and analysis of soil

extracts represented a major component of the method validation effort. Three different extraction solvents were examined: deionized (DI) water , potassium chloride, and methanol (Table 17).

7.3.2 Extraction Efficiency

DI water was evaluated because of its effectiveness in stripping ions from soils by establishing a severe concentration gradient between soil particles and the aqueous phase. A solution of 3M potassium chloride is a common soil extractant for anions. This solution can be effective in displacing adsorbed anions with potassium. Since HAN and TEAN are soluble in alcohols, methanol was also considered to be a potential extracting solvent. Analytical interferences caused by the solvents were examined using method blanks but were not observed.

Deionized water provided the best recovery of the extraction solvents tested (Table 17). However, inability to recover low concentrations of HAN and TEAN from clay and organic soil suggested that these compounds were either not stable or resisted extraction due to chemical interactions with soil particles. Table 18 indicates the results of extracting HAN and TEAN with DI water from soil immediately after spiking. Immediate extraction gave much better recovery compared to samples refrigerated for seven days. These results supported the hypothesis that HAN and TEAN were not stable in low concentrations in certain soil types.

To further investigate the effectiveness and efficiency of using DI water to extract HAN and TEAN, sandy, clayey, and organic soils were spiked with LGP and analyzed in triplicate. Each soil type was spiked with LGP to 2, 10, and 100 times the quantifiable limit for the soil accounting for dilution. Two sets of triplicate samples of spiked soil were prepared. One set was stored at 4°C for six days prior to aqueous extraction and the other was immediately extracted. The results shown in Tables 19 and 20 indicate that HAN and TEAN are not stable in clay and organic soil. Recovery of high concentration LGP spikes suggested that oxidatively reactive compounds in the soil were expended by less than the maximum amount of LGP added to the soil.

Extraction efficiency of DI water was determined using triplicate spiked samples. The added LGP was in contact with the soil matrix for six days at 4°C to facilitate adsorption. Each soil matrix was then extracted and the amount of HAN and TEAN recovered was compared to the

amount added. Variability among triplicate samples was also determined. The results shown in Table 19 indicate low recovery of low concentrations of both TEAN and HAN in each soil type. HAN recovery from sandy and organic soil was low at each concentration tested. Recovery from clayey soil increased with concentration. TEAN recovery exceeded 100 percent in several cases suggesting interference with TEAN detection due to soil specific interactions. Appendix B contains representative chromatograms developed during the analysis of soil.

8.0 Operation Observations and Summary

The WES method proved to be a useful method for the detection of LGP, ethanolamine, and diethanolamine. Aqueous samples have a one mg/L detection limit for both HAN and TEAN; however, a one mg/L quantifiable limit for TEAN was difficult to achieve on a routine basis. The quantifiable limit for TEAN typically ranged between one and three mg/L. Laboratory experience and conversations with WES scientists indicate that the WES method is complicated to reproduce and requires sophisticated detection equipment, a specific cation exchange column, a well controlled post-column chemical reaction to ionize target compounds, and skilled analysts to execute the method.

The method requires a relatively high level of maintenance to insure satisfactory performance. Empirical evidence indicates that a new cation exchange column will support the analysis of 300 to 400 samples before performance degrades. Attempts to restore a used column have not been successful to date. The detection electrode requires maintenance once or twice per week depending on the number of samples analyzed. Specifically, the electrode must be rebuilt to replenish electrolyte solution, polish the gold working electrode, and replace the semi-permeable membrane separating the reference electrode, sample chamber, and working electrode. The highly active surface of the column can attract constituents of the environmental water and soil samples. These strongly adsorbed components accumulate on the column lowering resolution and shortening the life of the column. The short life of the cation exchange column and the complex maintenance requirements of the electrode make the method challenging to reproduce and perform on a routine basis.

Matrix interferences were observed with the large concentrations of sodium in the seawater samples but were not observed with the small concentrations of ions in the nutrient broth. Reproducibility was poor in the lower concentrations of HAN and TEAN for every environmental matrix. This is probably due to the fluctuations of HAN and especially TEAN around the detection limit.

Extraction of HAN and TEAN from the soil proved to be difficult. Deionized water was the only promising extraction solvent identified. The poor recoveries in the clay and organic soil suggest a instability of the compounds or chemical interactions with the soil particles. Recovery from clayey soil increased with increasing concentration but exceeded 100 percent at the highest concentrations suggesting interactions with soil particles. The longer the LGP components were in contact with the soil particles the worse the recoveries were.

The WES method has been tested by evaluating its ability to quantitate HAN and TEAN in a variety of aqueous and soil matrices. Several difficulties noted during the method validation process are summarized below:

- The electrochemical cell used to detect HAN and TEAN required frequent maintenance;
- The performance of the chromatographic column began to deteriorate after 300 to 400 samples;
- Ionic interactions between dissolved minerals in groundwater appeared to cause changes in the detectability of TEAN resulting in larger measurements than expected;
- Low concentrations of HAN were not stable in groundwater and sea water;
- High concentrations of HAN in seawater were characterized by poor peak resolution;
- Recovery of low concentrations if HAN and TEAN from soil samples was very poor suggesting that low concentrations of HAN and TEAN were not stable in the presence of soil;
- Recovery of higher concentrations of HAN and TEAN from different soil types was variable and influenced by the soil type.

In spite of the difficulties listed above, the method delivered lower practical quantitation limits than other available methods. Background interference in untreated samples was generally very low or nondetectable. However, interactions between LGP and the sample

matrix often produced unexpected results. Nevertheless, the results indicate that the method will detect HAN and TEAN if they are present. Accuracy becomes problematic due to interactions between LGP components and the sample matrix.

The method will provide the acceptable precision to determine the biodegradability of HAN and TEAN since relative changes can be used to quantitate HAN and TEAN biodegradation. Care will be exercised to select test matrices that will result in the least amount of interference. This approach will help reduce the confounding effects of sample interference and chemical instability on the evaluation of biodegradation. With proper attention to QA and column and detector maintenance, the method is expected be useful during the LGP biodegradation study.

Table 1

Calibration Standards Used to Determine Quantifiable Ranges and Detection Limits

IT Project No. 322240

Description	Composition	Concentrations (mg/L)					
TEAN		400	150	50	10	1	
HAN		150	75	25	10	1	
Ethanolamine		30	25	10	1	0.5	0.25
Diethanolamine		75	50	25	10	1	0.5
LGP	HAN	261.7	86.4	43.2	21.6	8.7	4.3
	TEAN	81.5	26.9	13.5	6.7	2.7	1.3
Calibration Mix	HAN	150	75	15	7.5	1.5	
	TEAN	400	200	40	20	4	
	Diethanolamine	75	37.5	7.5	3.75	0.75	
	Ethanolamine	25	12.5	2.5	1.25	0.25	

Table 2

Calibration Series Run with Samples

IT Project No. 322240

Calibration Mix	Concentration (mg/L)						
HAN	118.4	59.2	29.6	14.8	7.4	3.7	1.48
TEAN	98.4	49.2	24.6	12.3	6.15	3.08	1.23

Table 3

Quality Assurance Samples Analyzed During Water Analysis

IT Project No. 322240

Approximate Sample Position	TEAN			HAN		
	Standard (mg/L)	Observed (mg/L)	Recovery	Standard (mg/L)	Observed (mg/L)	Recovery
10th Sample in Series	49.2	48	98%	59.2	58.6	99%
20th Sample in Series	6.15	6.7	109%	7.4	6.7	91%
Mean Recovery			103%			95%
Precision (±)			8%			6%

Table 4

Quality Assurance Samples Analyzed During Extraction Tests

IT Project No. 322240

Approximate Sample Position	TEAN			HAN		
	Standard (mg/L)	Observed (mg/L)	Recovery	Standard (mg/L)	Observed (mg/L)	Recovery
10th Sample in Series	6.15	7	114%	7.4	8.1	109%
20th Sample in Series	6.15	6.8	111%	7.4	7.1	96%
30th Sample in Series	12.3	11.1	90%	14.8	12.7	86%
Mean Recovery			105%			97%
Precision (±)			12%			12%

Table 5

Quality Assurance Samples Analyzed During Immediate Soil Extraction Tests

IT Project No. 322240

Approximate Sample Position	TEAN			HAN		
	Standard (mg/L)	Observed (mg/L)	Recovery	Standard (mg/L)	Observed (mg/L)	Recovery
10th Sample in Series	24.6	23.7	96%	29.6	28.5	96%
20th Sample in Series	6.15	6.5	106%	7.4	6.4	86%
30th Sample in Series	12.3	10.6	86%	14.8	12.6	85%
Mean Recovery			96%			89%
Precision (±)			10%			7%

Table 6

Quality Assurance Samples Analyzed During Six Day Adsorption Soil Extraction Tests

IT Project No. 322240

Approximate Sample Position	TEAN			HAN		
	Standard (mg/L)	Observed (mg/L)	Recovery	Standard (mg/L)	Observed (mg/L)	Recovery
10th Sample in Series	6.15	5.4	88%	7.4	7.2	97%
20th Sample in Series	12.3	18.3	149%	14.8	13.6	92%
30th Sample in Series	24.6	44.1	179%	29.6	28.8	97%
Mean Recovery			139%			95%
Precision (±)			34%			3%

Table 7

Method Precision for Groundwater and Seawater Samples

IT Project No. 322240

	TEAN (mg/L)			HAN (mg/L)		
Known Concentration	2	10	100	6.4	32.3	321.5
Measured Concentration	2.3	12.6	126	4.5	22	291
Measured Concentration	2.2	12.5	153	3.9	20.6	293
Measured Concentration	2.6	13	130	4	21	310
Measured Concentration	3	13.4	137	0	0	289
Measured Concentration	4.1	12.1	125	0	0	295
Measured Concentration	3.9	11.2	114	0	0	287
Mean	3.0	12.5	130.8	2.1	10.6	294.2
Standard Deviation	0.8	0.8	13.2	2.3	11.6	8.3
Recovery	151%	125%	131%	32%	33%	91%
Precision (±)	27%	6%	10%	110%	110%	3%

Table 8

Method Precision for Groundwater Samples

IT Project No. 322240

	TEAN [a] (mg/L)			HAN (mg/L)		
Known Concentration	2	10	100	6.4	32.3	322
Measured Concentration	2.3	12.6	126	4.5	22	291
Measured Concentration	2.2	12.5	153	3.9	20.6	293
Measured Concentration	2.6	13	130	4	21	310
Mean	2.4	12.7	136.3	4.1	21.2	298
Standard Deviation	0.2	0.3	14.6	0.3	0.7	10.4
Recovery	118%	127%	136%	65%	66%	93%
Precision (±)	9%	2%	11%	8%	3%	4%

[a] Background interference equivalent to 1.94 mg/L was subtracted from TEAN measurements

Table 9

Method Precision for Seawater Samples

IT Project No. 322240

	TEAN (mg/L)			HAN (mg/L)		
Known Concentration	2	10	100	6.4	32.3	321.5
Measured Concentration	3	13.4	137	0	0	289
Measured Concentration	4.1	12.1	125	0	0	295
Measured Concentration	3.9	11.2	114	0	0	287
Mean	3.7	12.2	125.3	0.0	0.0	290.3
Standard Deviation	0.6	1.1	11.5	0.0	0.0	4.2
Recovery	183%	122%	125%	0%	0%	90%
Precision (±)	16%	9%	9%	0%	0%	1%

Table 10

Method Precision for Sandy Soil Samples

IT Project No. 322240

	TEAN (mg/L)			HAN (mg/L)		
Known Concentration	4.94	49.4	494	15.86	158.6	1586
Measured Concentration	12	65	637	0	82	1602
Measured Concentration	7.7	62.9	664	0	90.9	1632
Measured Concentration	8.7	64.7	809	4	96.8	1744
Mean	9.5	64.2	703.3	1.3	89.9	1659.3
Standard Deviation	2.3	1.1	92.5	2.3	7.5	74.8
Recovery	192%	130%	142%	8%	57%	105%
Precision (±)	24%	2%	13%	173%	8%	5%

Table 11

Method Precision for Organic Soil Samples

IT Project No. 322240

	TEAN (mg/L)			HAN (mg/L)		
Known Concentration	4.94	49.4	494	15.86	158.6	1586
Measured Concentration	10.6	41.0	415	5.4	5.7	14.2
Measured Concentration	6.9	30.5	410	5.4	4.1	11.6
Measured Concentration	10.3	41.4	438	4	6.5	9.9
Mean	9.3	37.6	421	4.9	5.4	11.9
Standard Deviation	2.1	6.2	14.9	0.8	1.2	2.2
Recovery	188%	76%	85%	31%	3%	1%
Precision (±)	22%	16%	4%	16%	22%	18%

Table 12

Method Precision for Clayey Soil Samples

IT Project No. 322240

	TEAN (mg/L)			HAN (mg/L)		
Known Concentration	15.96	79.8	798	51.24	256.2	2562
Measured Concentration	0	62.8	959	18	233	4058
Measured Concentration	0	60	1085	24.2	229	3956
Measured Concentration	0	63	915	20.1	224	3725
Mean	0.0	61.9	986.3	20.8	228.7	3913.0
Standard Deviation	0.0	1.7	88.2	3.2	4.5	170.6
Recovery	0%	78%	124%	41%	89%	153%
Precision (±)	0%	3%	9%	15%	2%	4%

Table 13

Practical Quantitation Limits for HAN, TEAN, Ethanolamine, and Diethanolamine

IT Project No. 322240

Compound	Lower Quantifiable Limit (mg/L)	Upper Quantifiable Limit (mg/L)
HAN	1	150
TEAN	1	400
Ethanolamine	0.25	25
Diethanolamine	0.5	75

Table 14

Recovery of TEAN and HAN from LGP in Two Percent Nutrient Broth

IT Project No. 322240

Actual TEAN (mg/L)	TEAN Observed[a] (mg/L)	Percent Recovered	Actual HAN (mg/L)	HAN Observed[a] (mg/L)	Percent Recovered
67.9	64.9	96	218	213	98
27.2	32.1	118	87	103	118
6.79	6.68	98	21.8	20.7	95
2.72	1.6	59	8.7	5.1	59
1.36	0.8	59	4.4	1.6	36

[a]Observed values are adjusted for concentrations found in the nutrient broth blank; 2.99 mg/L HAN and 2.87 mg/L TEAN.

Table 15

Recovery of TEAN and HAN in Triplicate Groundwater Samples

TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
2	2.3	2.2	2.6	2.4	0.208	120
10	12.6	12.5	13.0	12.7	0.265	127
100	126	153	130	136	14.6	136
HAN Spike						
6.4	4.5	3.9	4.1	4.2	0.31	66
32.3	22	20.6	21.0	21.2	0.72	66
321.5	291	293	310	298	10.4	93

Table 16

Recovery of TEAN and HAN in Triplicate Seawater Samples (Results in mg/L)

IT Project No. 322240

TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
2	3.0	4.1	3.9	3.7	0.59	185
10	13.4	12.1	11.2	12.2	1.11	122
100	137	125	114	125	11.5	125
HAN Spike						
6.4	ND	ND	ND	ND	NA	0
32.3	ND	ND	ND	ND	NA	0
321.5	289	295	287	290	4.16	90

Table 17

Extraction of LGP from Soil Matrices After Seven Days Incubation With Different Extraction Solvents

IT Project No. 322240

DI Water Extraction	HAN-Actual (mg/kg)	HAN-Observed (mg/kg)	Percent Recovered	TEAN-Actual (mg/kg)	TEAN-Observed (mg/kg)	Percent Recovered
Clay	6.4	ND	0	2	ND	0
	32.3	4.1	13	10	ND	0
	321	200	62	100	82.9	83
Sand	6.4	3.63	57	2	9.1	455
	32.3	ND	0	10	16.9	169
	321	ND	0	100	0	0
Organic	6.4	1.9	30	2	ND	0
	32.3	1.6	5	10	12	120
	321	4.3	1	100	89.6	90
Potassium Chloride Extraction	HAN-Actual (mg/kg)	HAN-Observed (mg/kg)	Percent Recovered	TEAN-Actual (mg/kg)	TEAN-Observed (mg/kg)	Percent Recovered
Clay	6.4	ND	0	2	ND	0
	32.3	ND	0	10	ND	0
	321	ND	0	100	ND	0
Sand	6.4	ND	0	2	ND	0
	32.3	ND	0	10	ND	0
	321	ND	0	100	ND	0

Table 17 (continued)

Extraction of LGP from Soil Matrices After Seven Days Incubation With Different Extraction Solvents

IT Project No. 322240

Potassium Chloride Extraction	HAN-Actual (mg/kg)	HAN-Observed (mg/kg)	Percent Recovered	TEAN-Actual (mg/kg)	TEAN-Observed (mg/kg)	Percent Recovered
Organic	6.4	ND	0	2	ND	0
	32.3	ND	0	10	ND	0
	321	ND	0	100	ND	0
Methanol Extraction	**HAN-Actual (mg/kg)**	**HAN-Observed (mg/kg)**	**Percent Recovered**	**TEAN-Actual (mg/kg)**	**TEAN-Observed (mg/kg)**	**Percent Recovered**
Clay	6.4	ND	0	2	ND	0
	32.3	ND	0	10	ND	0
	321	10	3	100	ND	0
Sand	6.4	ND	0	2	ND	0
	32.3	ND	0	10	ND	0
	321	ND	0	100	ND	0
Organic	6.4	ND	0	2	7.6	280
	32.3	ND	0	10	11.4	114
	321	ND	0	100	91.9	92

Table 18

Immediate Extraction of LGP from Soil Matrices with Deionized Water

IT Project No. 322240

	HAN-Actual (mg/kg)	HAN - Observed (mg/kg	Percent Recovered	TEAN-Actual (mg/kg)	TEAN-Observed (mg/kg)	Percent Recovered
Clay	25.62	17	65	7.98	ND	0
	256.2	173	67	79.8	62	78
	2562	2,875	112	798	795	99.6
Sand	15.86	ND	0	4.94	ND	0
	158.6	67	42	49.4	43.3	88
	1,586	1,169	74	494	430	87
Organic	15.86	ND	0	4.94	ND	0
	158.6	ND	0	49.4	28.9	59
	1,586	49	3	494	351	71

Table 19

LGP Extraction from Soil Matrices with Deionized Water Six Days After Spiking (Results in mg/kg)

IT Project No. 322240

TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
CLAY SOIL						
15.96	0	0	0	0	0	0
79.8	55	123	123	100	39.3	125
798	1683	1770	1731	1728	43.6	216
HAN Spike						
51.24	0	10.2	12.2	7.5	6.5	15
256.2	167	201	186	184.7	17	72
2562	3492	3609	3558	3553	58.7	139
SANDY SOIL						
TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
4.94	NA[a]	0	0	0	0	0
49.4	51	60	48.6	53.2	6.0	108
494	662	708	670	680	24.6	138
HAN Spike						
15.86	NA[a]	0	0	0	0	0
158.6	0	0	0	0	0	0
1586	209	676	220	368	267	23

Table 19 (continued)

LGP Extraction from Soil Matrices with Deionized Water Six Days After Spiking (Results in mg/kg)

IT Project No. 322240

ORGANIC SOIL						
TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
4.94	0	0	0	0	0	0
49.4	12.5	40	26.1	26.2	13.8	53
494	414	455	475	448	31.1	91
HAN Spike						
15.86	3.8	0	0	1.27	---	8
158.6	0	0	4.4	1.5	---	0.9
1586	0	0	0	0	0	0

[a]NA, not analyzed, vial cracked during extraction.

Table 20

Immediate LGP Extraction from Soil Matrices Using Deionized Water (Results in mg/kg)

IT Project No. 322240

TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
CLAY SOIL						
15.96	0	0	0	0	0	0
79.8	62.8	60	63	61.9	1.7	78
798	959	1085	915	986	88.2	124
HAN Spike						
51.24	18	24.2	20.1	20.8	3.2	41
256.2	233	229	224	229	4.5	89
2562	4058	3956	3725	3913	171	153
SANDY SOIL						
TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
4.94	12	7.7	8.7	9.5	2.3	192
49.4	65	62.9	64.7	64.2	1.1	130
494	673	664	809	715	81.2	145
HAN Spike						
15.86	0	0	4	103	2.3	8
158.6	82	90.9	96.8	89.9	7.5	56.7
1586	1602	1632	1744	1659	74.8	105

Table 20 (continued)

Immediate LGP Extraction from Soil Matrices Using Deionized Water (Results in mg/kg)

IT Project No. 322240

ORGANIC SOIL						
TEAN Spike	A	B	C	Average	Standard Deviation	Percent Recovered
4.94	10.6	6.9	10.3	9.3	2.1	188
49.4	41	30.5	41.4	37.6	6.2	76
494	415	410	438	421	14.9	85
HAN Spike						
15.86	5.4	5.4	4	4.9	0.8	31
158.6	5.7	4.1	6.5	5.4	1.2	3.4
1586	14.2	11.6	9.9	11.9	2.2	0.8

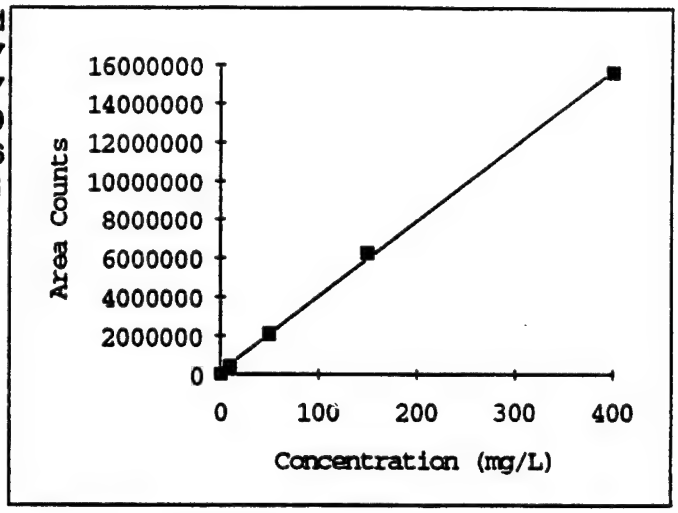
Appendix A

Linear Regression and Lack-of-Fit Testing on WES Method Calibration Data

WES Method Verification
USATHAMA, IT Project No. 322240

Regression Analysis of TEAN Analytical
24-Aug-93

Concentration	Area Count	Predicted
400	15618440	15729646.77
150	6263341	5967393.197
50	2082916	2062491.769
10	419919	500531.1976
1	24537	149090.0691



Regression Statistics

Multiple R	0.99963683
R Square	0.99927379
Adjusted R Square	0.99903171
Standard Error	201974.412
Observations	5

Analysis of Variance

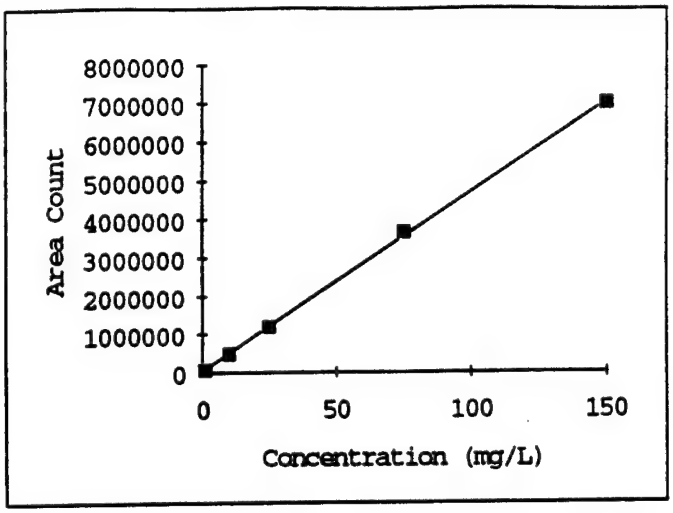
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	1.68397E+14	1.68397E+14	4128.0149	8.30769E-06	
Residual	3	1.22381E+11	40793663280			
Total	4	1.68519E+14	4.21298E+13			
LOF	-1	-1.68397E+14	1.68397E+14	3.9970951	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	110041.055	116938.8522	0.941013639	0.3999777	-262110.9124	482193.022
Concentration	39049.0143	607.770262	64.24962971	3.515E-07	37114.81624	40983.2123

Regression Analysis of HAN Analytical

24-Aug-93

Concentration	Area Count	Predicted
150	7001735	7059552.856
75	3676407	3549891.463
25	1190001	1210117.202
10	478796	508184.923
1	67833	87025.55589



Regression Statistics

Multiple R	0.99968546
R Square	0.99937102
Adjusted R Square	0.99916136
Standard Error	83637.7308
Observations	5

Analysis of Variance

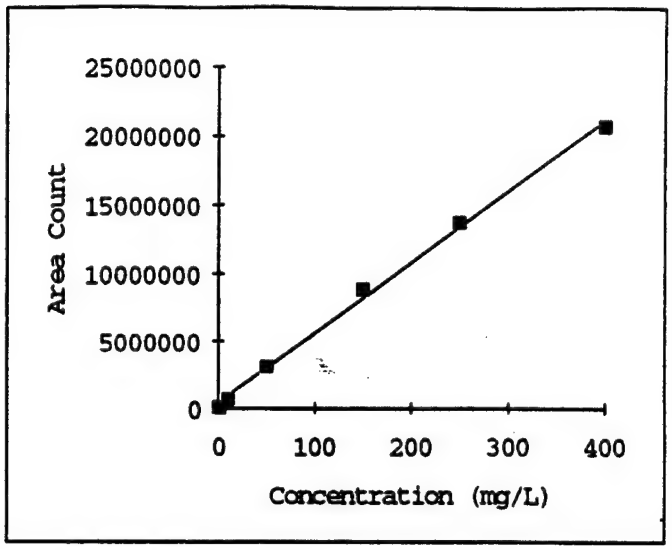
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	3.33439E+13	3.33439E+13	4766.6369	6.69614E-06	
Residual	3	20985810054	6995270018			
Total	4	3.33649E+13	8.34122E+12			
LOF	-1	-3.33439E+13	3.33439E+13	3.9974841	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	40230.0707	51486.49799	0.781371276	0.478245	-123623.0984	204083.24
Concentration	46795.4852	677.7943117	69.040835	2.637E-07	44638.43921	48952.5313

Regression Analysis of TEAN Analytical

31-Aug-93

Concentration	Area Count	Predicted
400	20716454	21183767.51
250	13729954	13387513.7
150	8854707	8190011.165
50	3082794	2992508.628
10	660929	913507.6132
1	68203	445732.3849



Regression Statistics

Multiple R	0.99852322
R Square	0.99704861
Adjusted R Square	0.99631077
Standard Error	497981.843
Observations	6

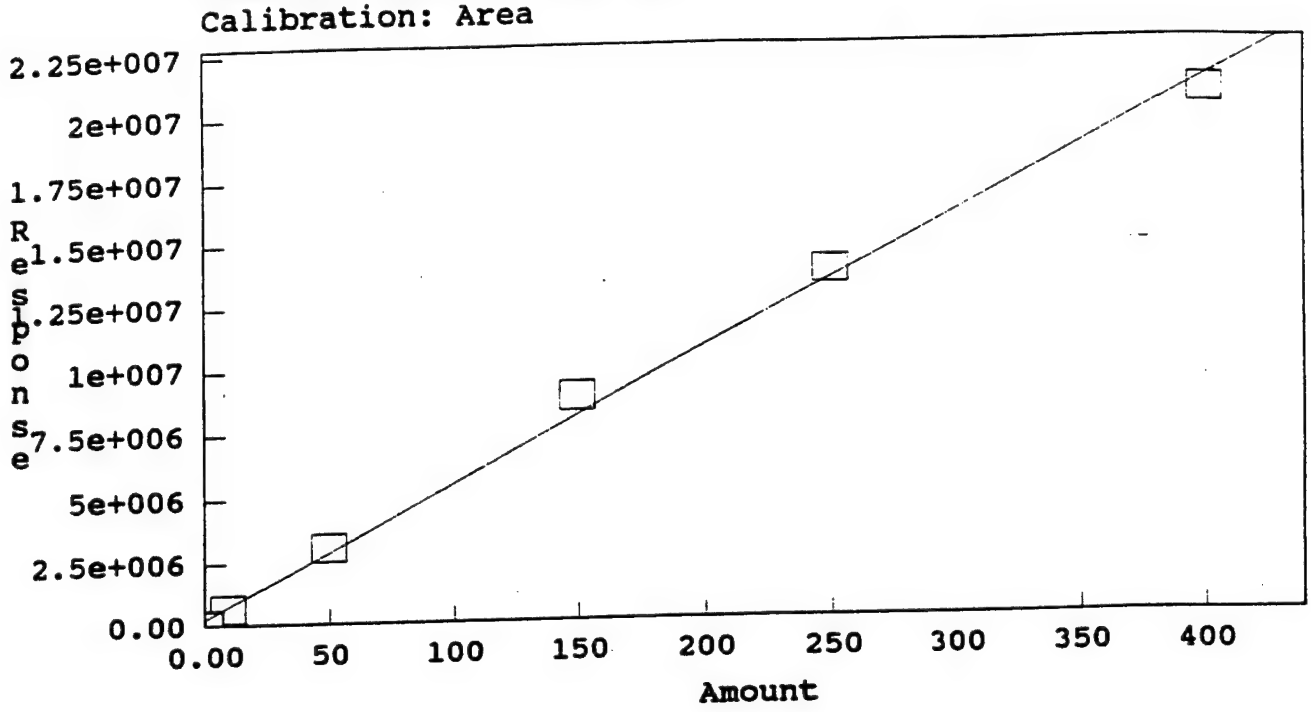
Analysis of Variance

	df	Sum of Squares	Mean Square	F	Significance F	F[0.05(1,4)]
Regression	1	3.35102E+14	3.35102E+14	1351.2958	3.26972E-06	
Residual	4	9.91944E+11	2.47986E+11			
Total	5	3.36094E+14	6.72189E+13			
LOF	-1	-3.35102E+14	3.35102E+14	4.9852431	Significant Fit	7.71

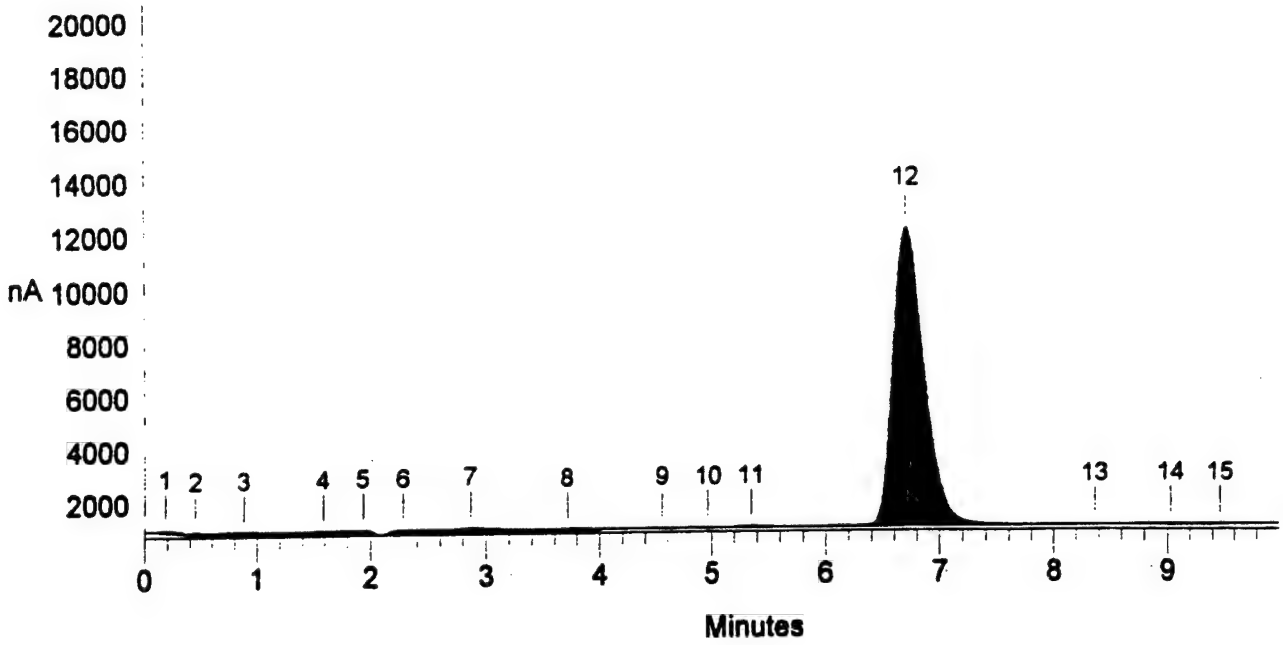
	Coefficients	Standard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	393757.36	287223.5678	1.370908949	0.2287374	-403704.761	1191219.48
Concentration	51975.0254	1413.902624	36.75997518	2.805E-07	48049.39422	55900.6565

-Method Updated: 13:27 on Tue, 31 Aug 1993

Component: TEAN
Fit Type: Linear
r^2 = 0.997158
Amt = Resp * 1.906e-005 + -5.317
Resp = Amt * 5.246e+004 + 2.789e+005
Standardization: External
Calibration: Area



File: wes83101.D02 Sample: AUTOCAL1



Sample Name: AUTOCAL1 Date: 08/31/1993 10:51:28
Data File : C:\DX\DATA\wes83101.D02
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 2 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
12	6.70	TEAN	400.000	1108906	20716454	1	0.00
		Totals	400.000	1108906	20716454		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.18		0.000	7085	84533	1	
2	0.45		0.000	4444	38260	2	
3	0.88		0.000	9262	373471	2	
4	1.58		0.000	16613	377567	2	
5	1.93		0.000	22220	380620	2	
6	2.28		0.000	20402	471069	2	
7	2.87		0.000	20304	671346	2	
8	3.72		0.000	5201	185506	2	
11	5.35		0.000	3542	48479	2	
14	9.03		0.000	1603	40048	2	
15	9.47		0.000	1198	30128	2	
		Totals	0.000	111874	2701027		

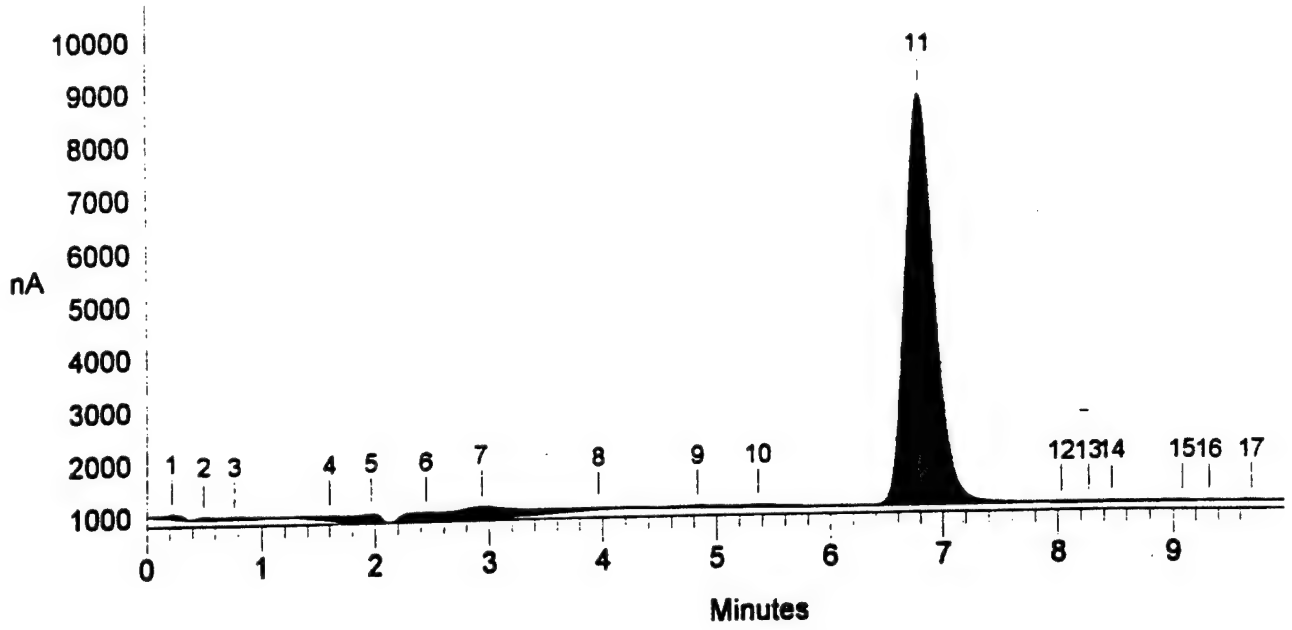
********** AUTOMATIC CALIBRATION UPDATE **********

Sample Name: AUTOCAL1 Date: Tue Aug 31 10:51:28 1993
Raw File : C:\DX\DATA\wes83101.D02
Method : C:\DX\METHOD\tean831.met Calibration Level: 1
ACI Address: 1 System : 1 Inject#: 2 Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.62	6.70	6.70	1.562e+007	2.072e+007	2.072e+007

File: wes83101.D03 Sample: AUTOCAL2



Sample Name: AUTOCAL2 Date: 08/31/1993 11:02:07
Data File : C:\DX\DATA\wes83101.D03
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 3 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
11	6.78	TEAN	250.000	777155	13729954	1	0.00
		Totals	250.000	777155	13729954		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.22		0.000	7825	91904	1	
2	0.50		0.000	3860	35307	2	
3	0.77		0.000	2435	44790	2	
4	1.60		0.000	8287	173985	2	
5	1.97		0.000	19242	286529	2	
6	2.45		0.000	19950	454930	2	
7	2.93		0.000	22760	979656	3	
9	4.83		0.000	2510	57241	2	
10	5.37		0.000	3256	55321	2	
14	8.47		0.000	913	16062	2	
15	9.08		0.000	1172	22757	2	
17	9.70		0.000	940	14946	2	
		Totals	0.000	93149	2233429		

********** AUTOMATIC CALIBRATION UPDATE **********

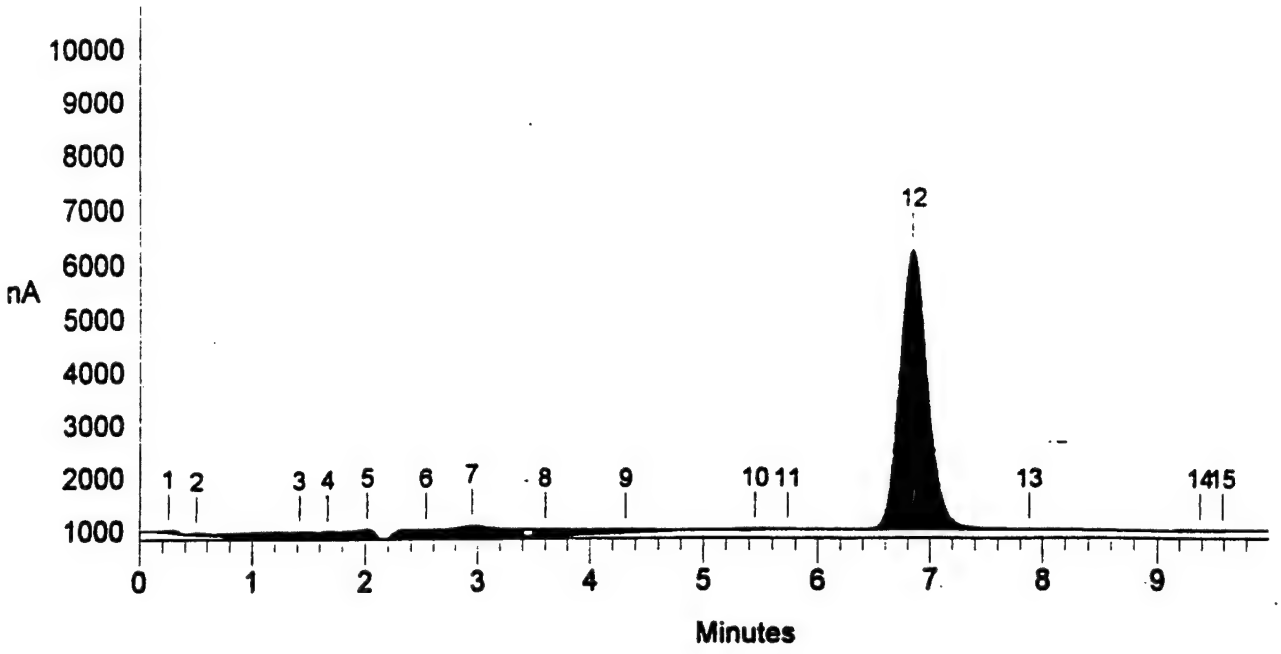
Sample Name: AUTOCAL2
Raw File : C:\DX\DATA\wes83101.D03
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System : 1 Inject#: 3

Date: Tue Aug 31 11:02:07 1993
Calibration Level: 2
Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.70	6.78	6.78	6.263e+006	1.373e+007	1.373e+007

File: wes83101.D04 Sample: AUTOCAL3



Sample Name: AUTOCAL3 Date: 08/31/1993 11:12:46
Data File : C:\DX\DATA\wes83101.D04
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 4 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
12	6.83	TEAN	150.000	522268	8854707	3	0.00
		Totals	150.000	522268	8854707		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.25		0.000	4241	37675	1	
2	0.50		0.000	2834	23247	2	
3	1.42		0.000	13708	479134	2	
4	1.67		0.000	16708	210370	2	
5	2.02		0.000	24606	388519	2	
6	2.53		0.000	21193	530966	2	
7	2.95		0.000	25431	902365	2	
8	3.60		0.000	12413	286784	2	
9	4.32		0.000	5927	245531	2	
10	5.45		0.000	2322	39958	2	
14	9.38		0.000	1256	22247	2	
		Totals	0.000	130639	3166797		

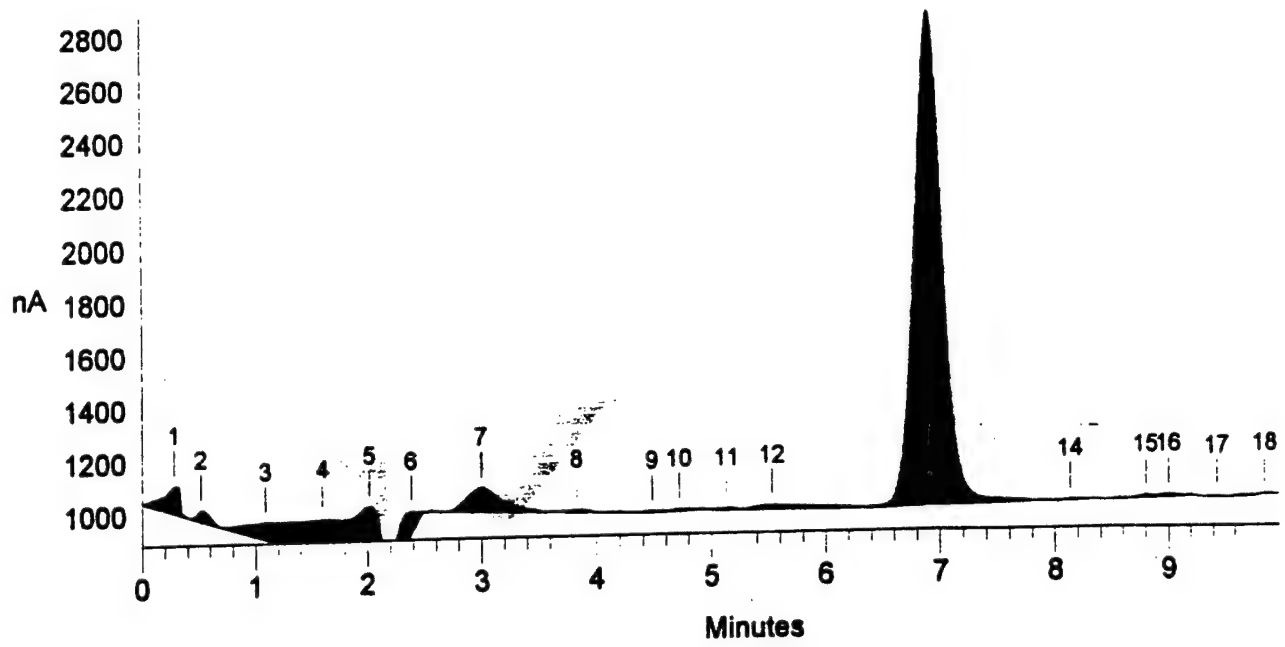
********** AUTOMATIC CALIBRATION UPDATE **********

Sample Name: AUTOCAL3 Date: Tue Aug 31 11:12:46 1993
Raw File : C:\DX\DATA\wes83101.D04
Method : C:\DX\METHOD\tean831.met Calibration Level: 3
ACI Address: 1 System : 1 Inject#: 4 Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.78	6.83	6.83	2.083e+006	8.855e+006	8.855e+006

File: wes83101.D05 Sample: AUTOCAL4



Sample Name: AUTOCAL4 Date: 08/31/1993 11:23:23
Data File : C:\DX\DATA\wes83101.D05
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 5 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
13	6.90	TEAN	50.000	186011	3082794	1	0.00
		Totals	50.000	186011	3082794		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.28		0.000	10447	113699	2	
2	0.52		0.000	4236	36436	2	
3	1.08		0.000	6728	136038	2	
4	1.60		0.000	14168	385396	2	
5	2.02		0.000	24035	440472	2	
6	2.38		0.000	8396	123753	1	
7	3.00		0.000	9138	192800	1	
8	3.83		0.000	982	11638	1	
12	5.53		0.000	1514	52328	1	
		Totals	0.000	79644	1492559		

********** AUTOMATIC CALIBRATION UPDATE **********

Sample Name: AUTOCAL4 Date: Tue Aug 31 11:23:23 1993
Raw File : C:\DX\DATA\wes83101.D05
Method : C:\DX\METHOD\tean831.met Calibration Level: 4
ACI Address: 1 System : 1 Inject#: 5 Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.83	6.90	6.90	4.199e+005	3.083e+006	3.083e+006

Sample Name: AUTOCAL5 Date: 08/31/1993 11:34:02
Data File : C:\DX\DATA\wes83101.D06
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 6 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

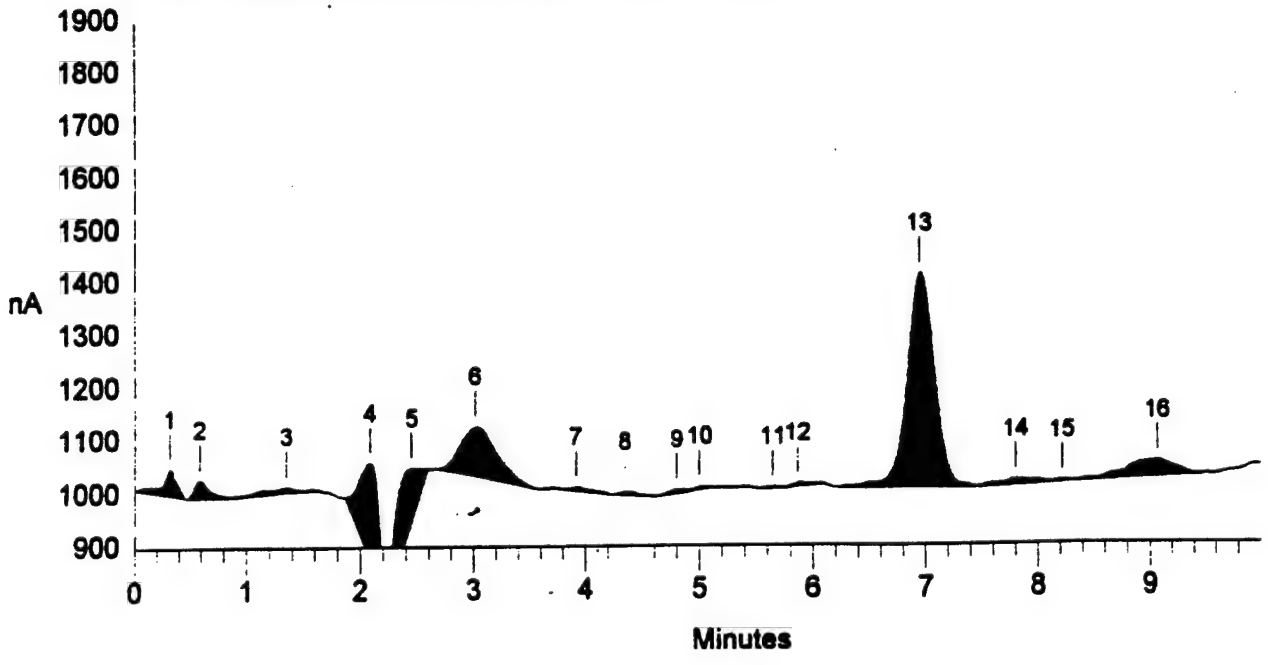
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
13	6.95	TEAN	10.000	40348	660929	1	0.00
		Totals	10.000	40348	660929		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.32		0.000	4922	44879	1	
2	0.58		0.000	3343	29447	1	
3	1.35		0.000	857	15574	1	
4	2.08		0.000	18320	209494	1	
5	2.45		0.000	9614	160554	1	
6	3.02		0.000	9315	206643	1	
14	7.80		0.000	987	19847	2	
16	9.07		0.000	3053	91277	2	
		Totals	0.000	50411	777715		

File: wes83101.D06 Sample: AUTOCAL5



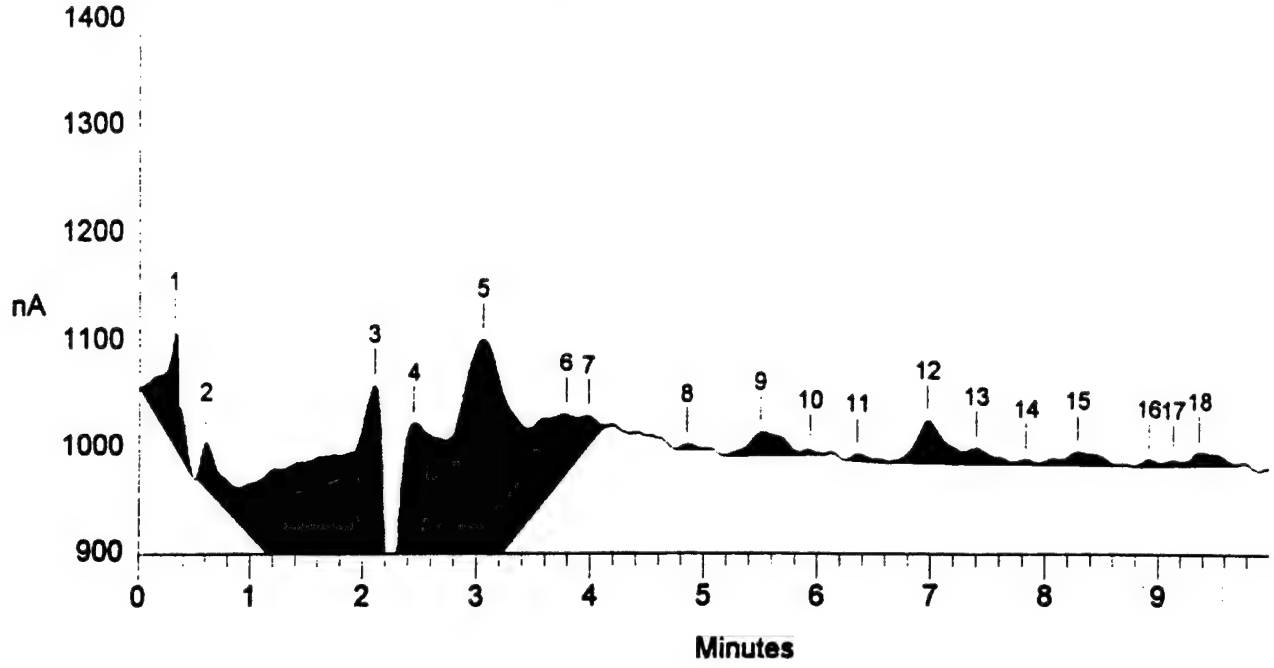
********** AUTOMATIC CALIBRATION UPDATE **********

Sample Name: AUTOCAL5 Date: Tue Aug 31 11:34:02 1993
Raw File : C:\DX\DATA\wes83101.D06
Method : C:\DX\METHOD\tean831.met Calibration Level: 5
ACI Address: 1 System : 1 Inject#: 6 Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.90	6.95	6.95	2.454e+004	6.609e+005	6.609e+005

File: wes83101.D07 Sample: AUTOCAL6



Sample Name: AUTOCAL6 Date: 08/31/1993 11:44:39
Data File : C:\DX\DATA\wes83101.D07
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 7 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
12	6.97	TEAN	1.000	3905	68203	2	0.00
		Totals	1.000	3905	68203		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.32		0.000	10623	112448	1	
2	0.60		0.000	4304	64950	2	
3	2.10		0.000	26298	1045548	2	
4	2.45		0.000	22324	511024	2	
5	3.05		0.000	22143	696808	2	
6	3.78		0.000	5514	172394	2	
7	3.98		0.000	2656	26559	2	
9	5.50		0.000	2107	44734	3	
13	7.40		0.000	1530	22299	2	
15	8.28		0.000	1286	27175	2	
18	9.37		0.000	1227	20101	2	
		Totals	0.000	100010	2744040		

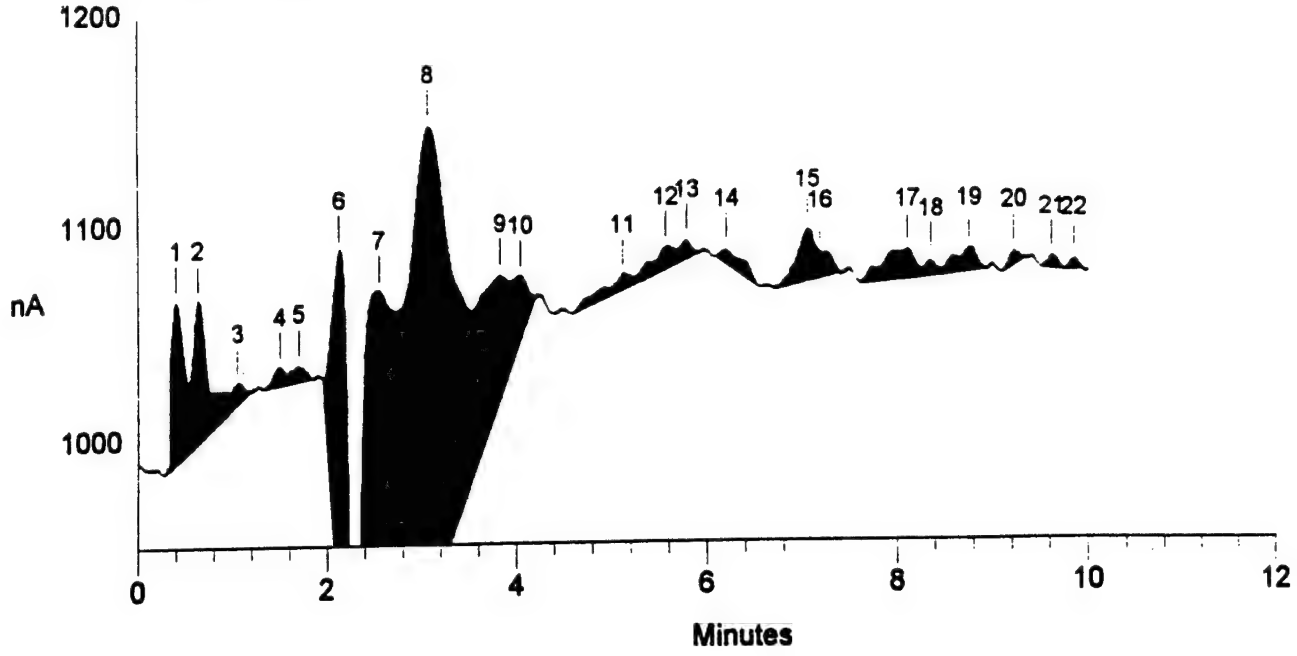
********** AUTOMATIC CALIBRATION UPDATE **********

Sample Name: AUTOCAL6 Date: Tue Aug 31 11:44:39 1993
Raw File : C:\DX\DATA\wes83101.D07
Method : C:\DX\METHOD\tean831.met Calibration Level: 6
ACI Address: 1 System : 1 Inject#: 7 Detector: PAD

********** COMPONENTS FOUND IN THIS RUN **********

COMP NUM	COMPONENT NAME	OLD RET.TIME	MEASURED RET.TIME	NEW RET.TIME	OLD RESPONSE	MEASURED RESPONSE	NEW RESPONSE
1	TEAN	6.95	6.97	6.97	7.151e+005	6.820e+004	6.820e+004

File: WES83101.D08 Sample: AUTOCAL7



Data Reprocessed On 08/31/1993 13:26:51

Sample Name: AUTOCAL7 Date: 08/31/1993 11:55:18
Data File : C:\DX\DATA\WES83101.D08
Method : C:\DX\METHOD\tean831.met
ACI Address: 1 System: 1 Inject#: 8 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	12.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
15	7.05	TEAN	0.500	2328	32979	2	0.00
		Totals	0.500	2328	32979		

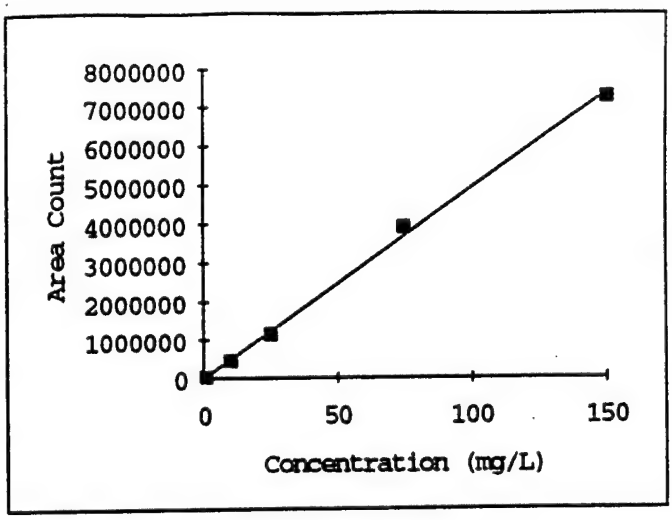
********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.40		0.000	7621	69445	2	
2	0.63		0.000	6595	74960	3	
6	2.13		0.000	18091	204639	1	
7	2.55		0.000	21287	454429	2	
8	3.07		0.000	22444	784856	2	
9	3.83		0.000	5724	149716	2	
10	4.05		0.000	2834	37741	2	
11	5.12		0.000	635	16215	2	
12	5.57		0.000	971	18309	2	
14	6.20		0.000	639	12061	1	
17	8.10		0.000	1296	33305	2	
19	8.75		0.000	1037	16991	2	
		Totals	0.000	89172	1872667		

Regression Analysis of HAN Analytical

31-Aug-93

Concentration	Area Count	Predicted
150	7269071	7365749.336
75	3905850	3681873.465
25	1149884	1225956.218
10	451019	489181.0433
1	34052	47115.93884



Regression Statistics

Multiple R	0.99909034
R Square	0.99818152
Adjusted R Square	0.99757535
Standard Error	149361.061
Observations	5

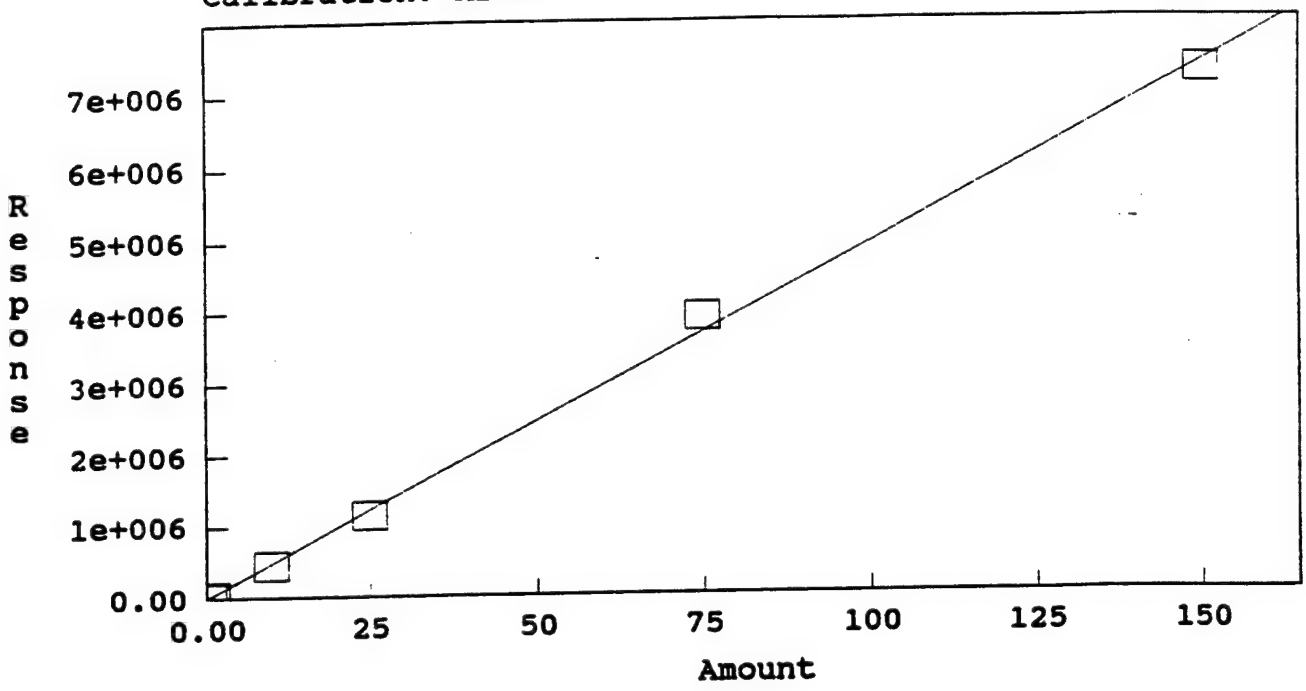
Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	3.67364E+13	3.67364E+13	1646.7259	3.29299E-05	
Residual	3	66926179245	22308726415			
Total	4	3.68033E+13	9.20082E+12			
LOF	-1	-3.67364E+13	3.67364E+13	3.9927261	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-2002.4061	91945.08111	-0.02177828	0.9836679	-294612.9643	290608.152
Concentration	49118.3449	1210.411572	40.57987058	2.204E-06	45266.2715	52970.4184

-Method Updated: 15:22 on Tue, 31 Aug 1993

Component: HAN
Fit Type: Linear
r^2 = 0.998413
Amt = Resp * 2.032e-005 + 0.161
Resp = Amt * 4.922e+004 + -7923
Standardization: External
Calibration: Area



Data Reprocessed On 08/31/1993 14:47:34

Sample Name: AUTOCAL1 Date: 08/31/1993 13:56:29
Data File : C:\DX\DATA\WES831X1.D03
Method : C:\DX\METHOD\han831.met
ACI Address: 1 System: 1 Inject#: 3 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

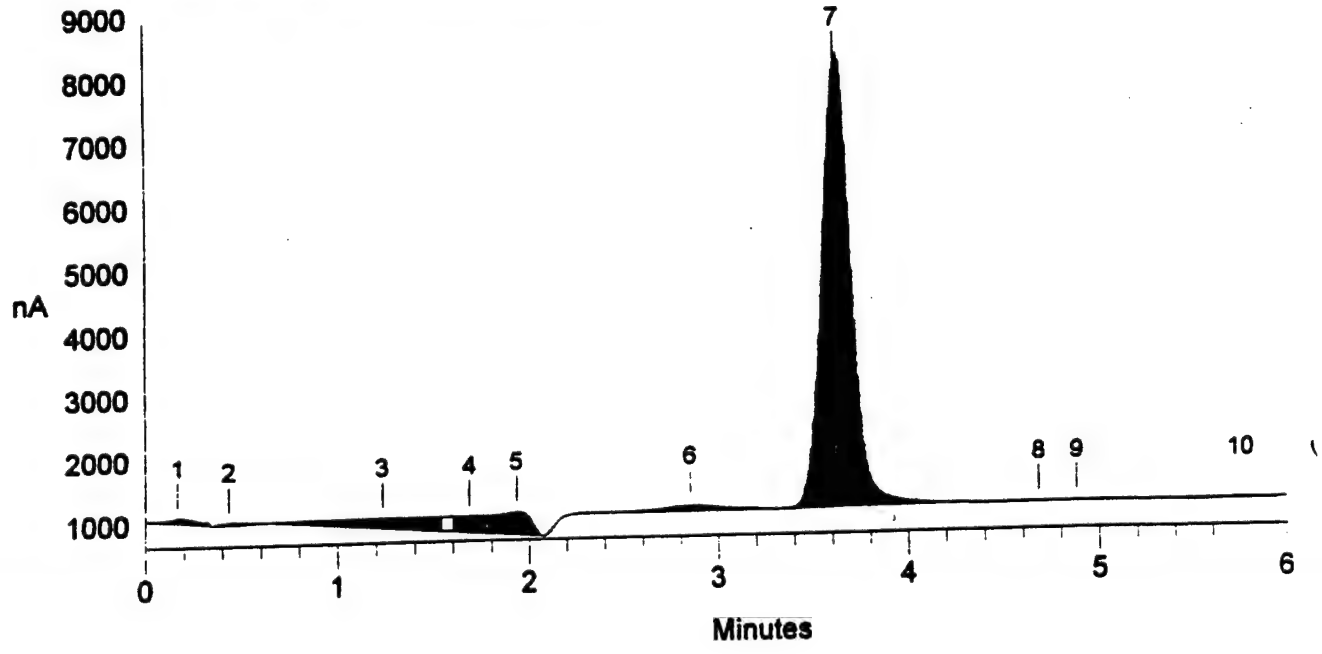
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
7	3.62	HAN	150.000	692063	7269071	1	0.00
		Totals	150.000	692063	7269071		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	6754	73877	1	
2	0.43		0.000	2721	18157	1	
3	1.23		0.000	15575	616595	2	
4	1.68		0.000	25685	258725	2	
5	1.93		0.000	36162	466236	2	
6	2.85		0.000	7292	144831	1	
		Totals	0.000	94189	1578420		

File: WES831X1.D03 Sample: AUTOCAL1



Data Reprocessed On 08/31/1993 15:15:14

Sample Name: AUTOCAL2 Date: 08/31/1993 14:03:05
Data File : C:\DX\DATA\WES831X1.D04
Method : C:\DX\METHOD\HAN831.MET
ACI Address: 1 System: 1 Inject#: 4 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

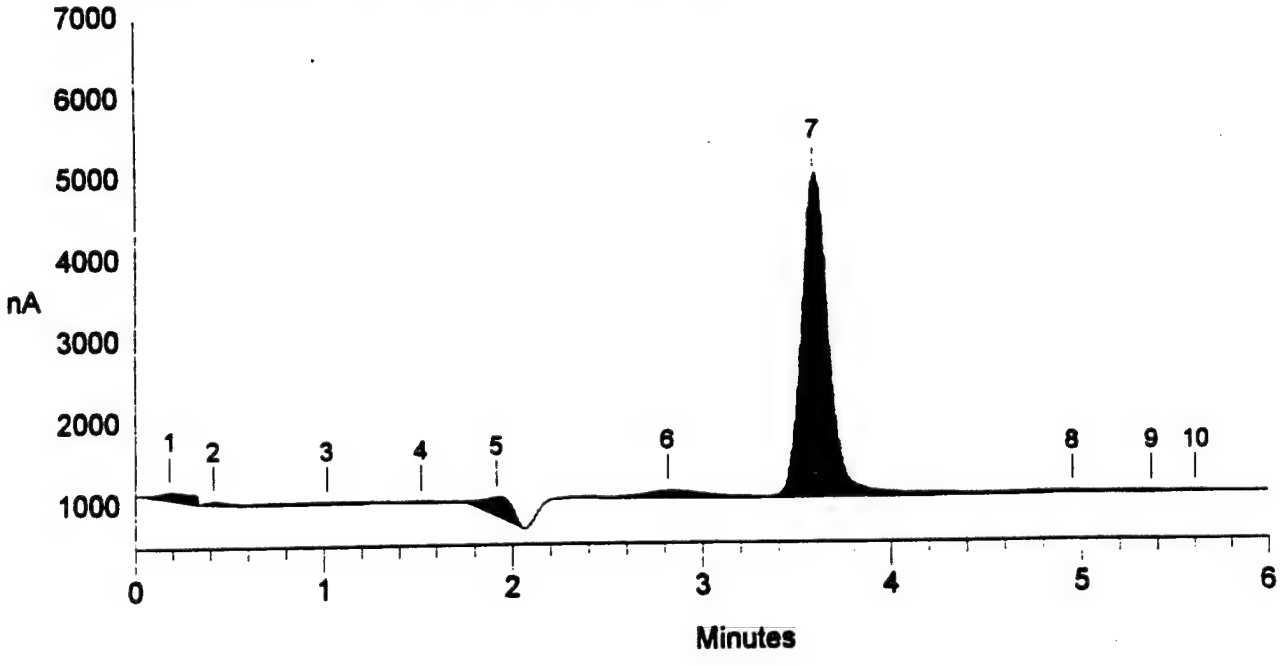
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
7	3.58	HAN	75.000	386402	3905850	3	0.00
		Totals	75.000	386402	3905850		

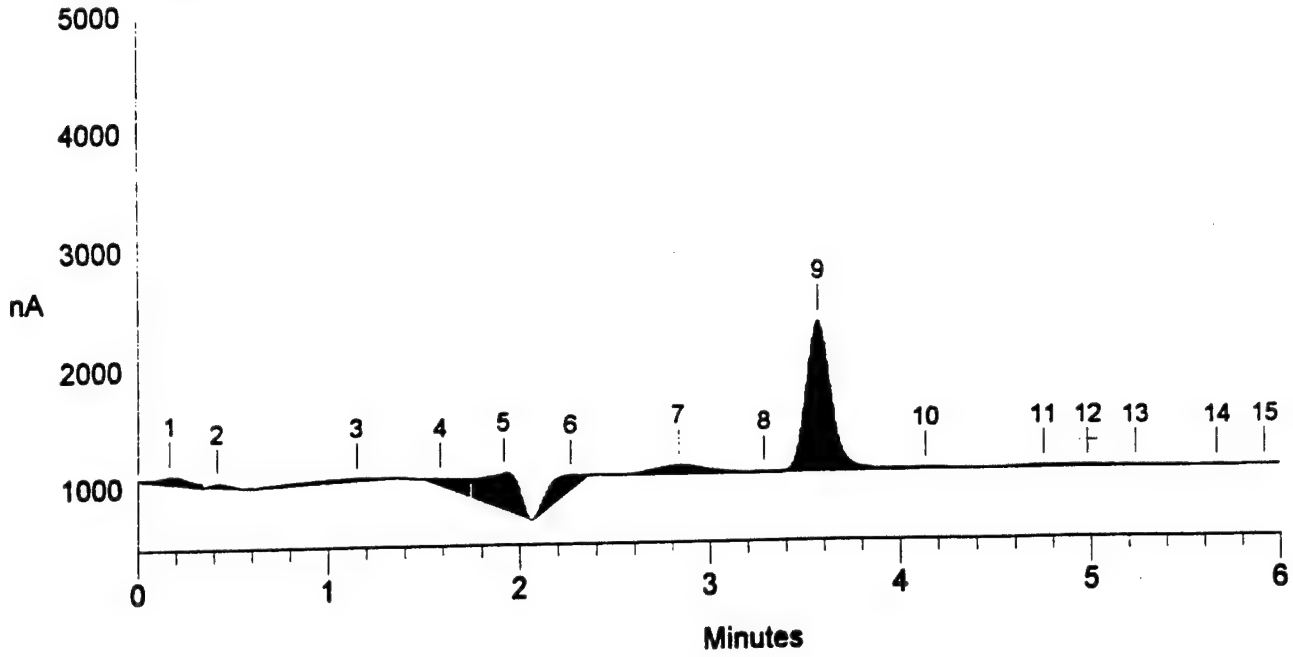
********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.18		0.000	8914	119901	1	
2	0.42		0.000	2681	18279	1	
3	1.02		0.000	646	15124	1	
5	1.92		0.000	23120	250851	1	
6	2.82		0.000	8052	154371	1	
		Totals	0.000	43413	558526		

File: WES831X1.D04 Sample: AUTOCAL2



File: WES831X1.D05 Sample: AUTOCAL3



Data Reprocessed On 08/31/1993 15:16:36

Sample Name: AUTOCAL3 Date: 08/31/1993 14:09:44
Data File : C:\DX\DATA\WES831X1.D05
Method : C:\DX\METHOD\HAN831.MET
ACI Address: 1 System: 1 Inject#: 5 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
9	3.57	HAN	25.000	126883	1149884	3	0.00
		Totals	25.000	126883	1149884		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	6218	72185	1	
2	0.42		0.000	2172	13433	1	
3	1.15		0.000	1178	38562	1	
4	1.58		0.000	5568	104980	2	
5	1.92		0.000	30819	403440	2	
6	2.27		0.000	11227	177360	1	
7	2.83		0.000	7131	131428	1	
11	4.75		0.000	1141	15508	2	
12	4.98		0.000	1086	13297	2	
		Totals	0.000	66539	970193		

Data Reprocessed On 08/31/1993 15:18:05

Sample Name: AUTOCAL4 Date: 08/31/1993 14:16:21
Data File : C:\DX\DATA\WES831X1.D06
Method : C:\DX\METHOD\HAN831.MET
ACI Address: 1 System: 1 Inject#: 6 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

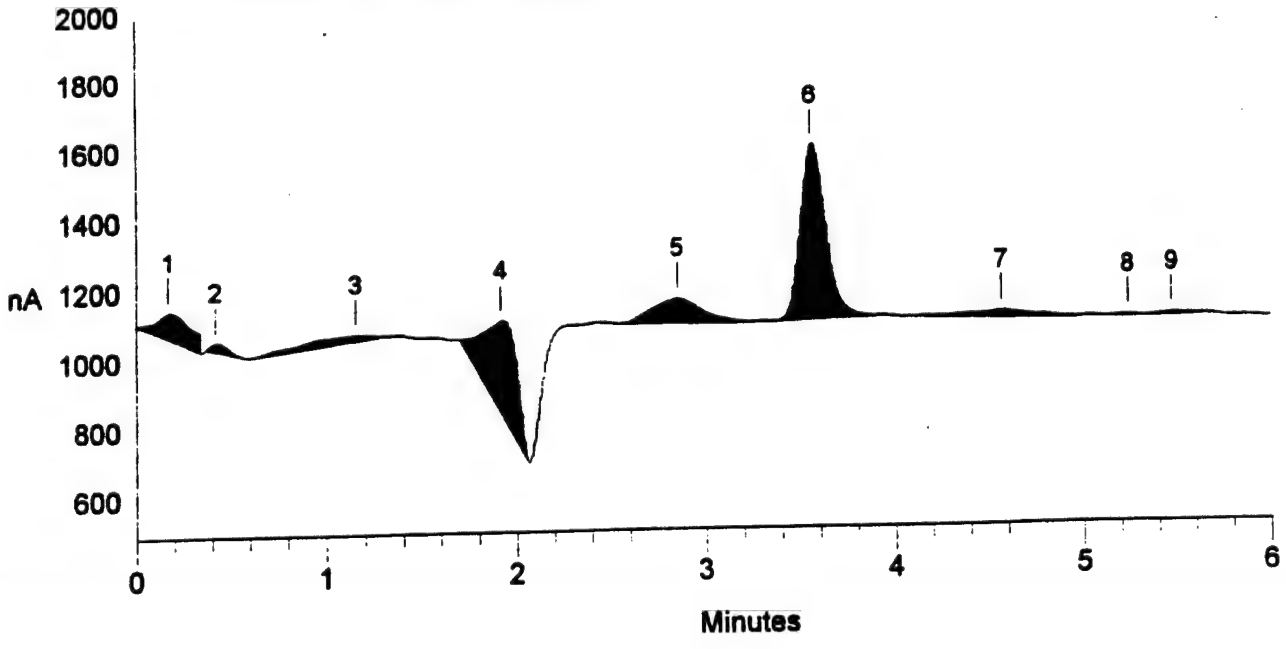
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
6	3.55	HAN	10.000	49594	451019	1	0.00
		Totals	10.000	49594	451019		

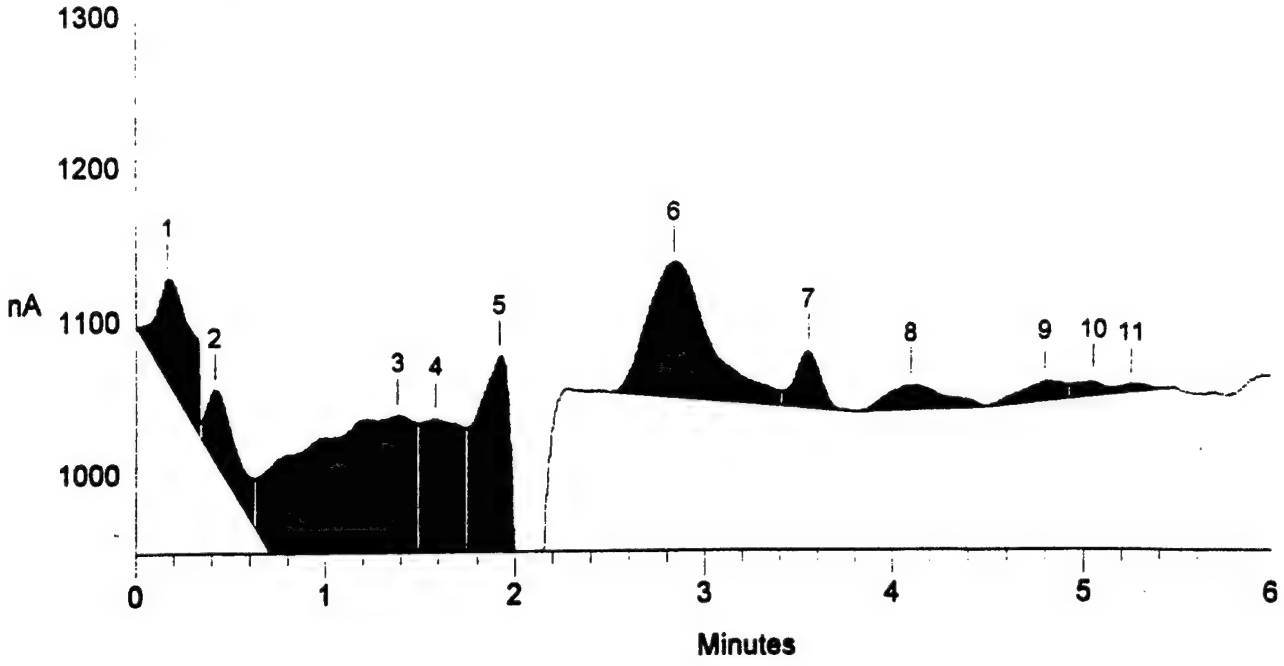
********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	7372	98593	1	
2	0.42		0.000	2327	15350	1	
3	1.15		0.000	1499	43914	1	
4	1.92		0.000	26157	322543	1	
5	2.85		0.000	6864	123096	1	
7	4.57		0.000	1847	41235	1	
		Totals	0.000	46066	644731		

File: WES831X1.D06 Sample: AUTOCAL4



File: WES831X1.D07 Sample: AUTOCAL5



Data Reprocessed On 08/31/1993 15:20:10

Sample Name: AUTOCAL5 Date: 08/31/1993 14:22:59
Data File : C:\DX\DATA\WES831X1.D07
Method : C:\DX\METHOD\HAN831.MET
ACI Address: 1 System: 1 Inject#: 7 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
7	3.55	HAN	1.000	3684	34052	2	0.00
		Totals	1.000	3684	34052		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	6533	90081	2	
2	0.42		0.000	4635	55149	2	
3	1.38		0.000	23312	767312	2	
4	1.58		0.000	27252	392635	2	
5	1.92		0.000	38226	554736	2	
6	2.83		0.000	8680	199505	2	
8	4.10		0.000	1586	32968	1	
9	4.80		0.000	1178	19660	2	
		Totals	0.000	111401	2112046		

Data Reprocessed On 08/31/1993 15:22:04

Sample Name: AUTOCAL6 Date: 08/31/1993 14:29:36
Data File : C:\DX\DATA\WES831X1.D08
Method : C:\DX\METHOD\HAN831.MET
ACI Address: 1 System: 1 Inject#: 8 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	1800	5Hz	0.00	6.00	10000

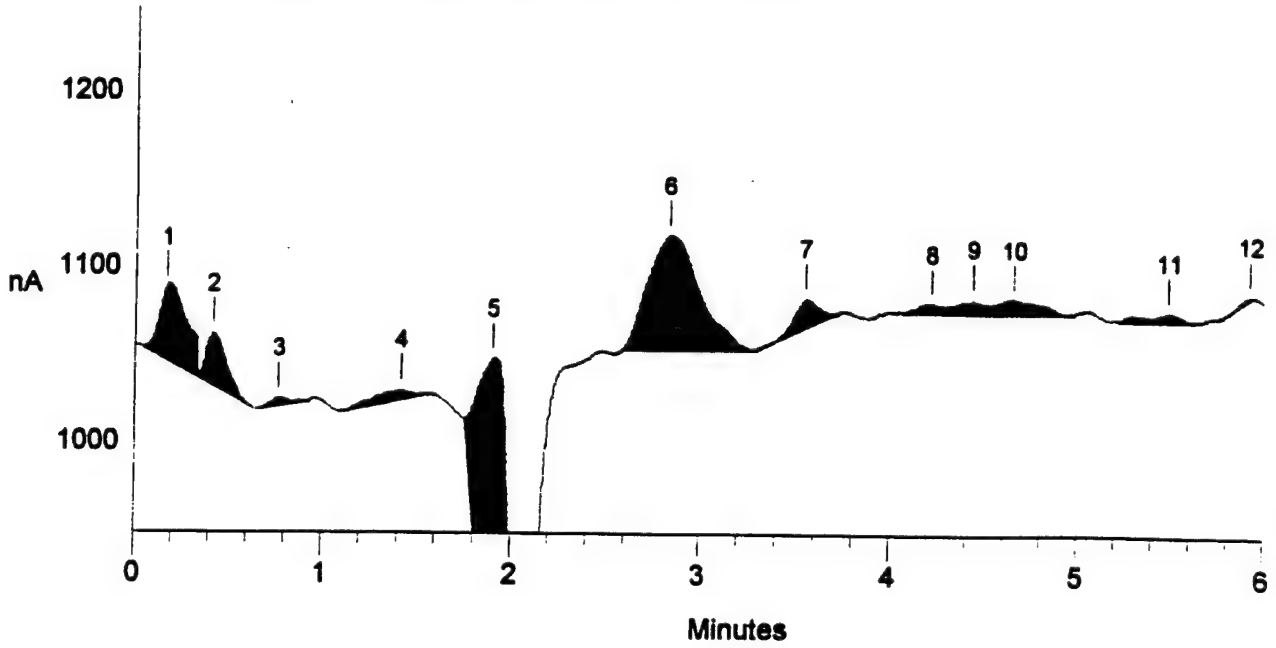
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
7	3.55	HAN	0.500	1616	13380	1	0.00
		Totals	0.500	1616	13380		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	4097	44854	2	
2	0.42		0.000	2943	21905	2	
5	1.90		0.000	21580	258000	1	
6	2.83		0.000	6519	124318	1	
10	4.65		0.000	805	12326	2	
		Totals	0.000	35945	461403		

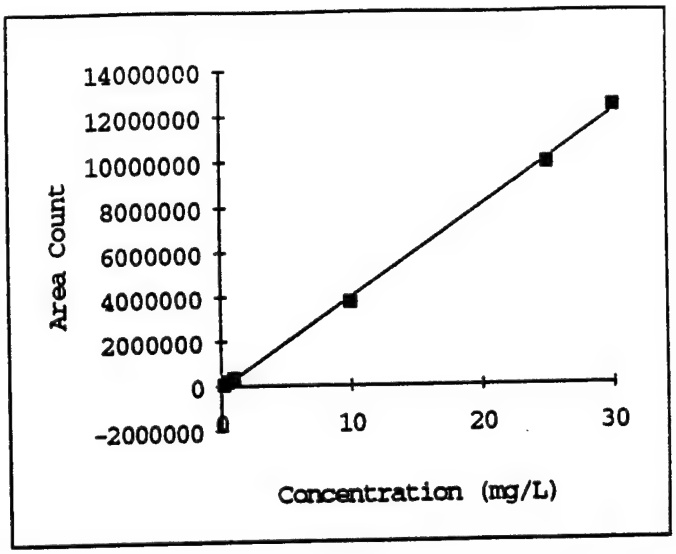
File: WES831X1.D08 Sample: AUTOCAL6



Regression Analysis of Ethanolamine Analytical

31-Aug-93

Concentration	Area Count	Predicted
30	12399178	12175583.88
25	9948542	10126654.11
10	3745812	3979864.802
1	302480	291791.2162
0.5	208290	86898.23919
0.25	40942	-15548.2493



Regression Statistics

Multiple R	0.99947971
R Square	0.99895969
Adjusted R Square	0.99869962
Standard Error	196558.087
Observations	6

Analysis of Variance

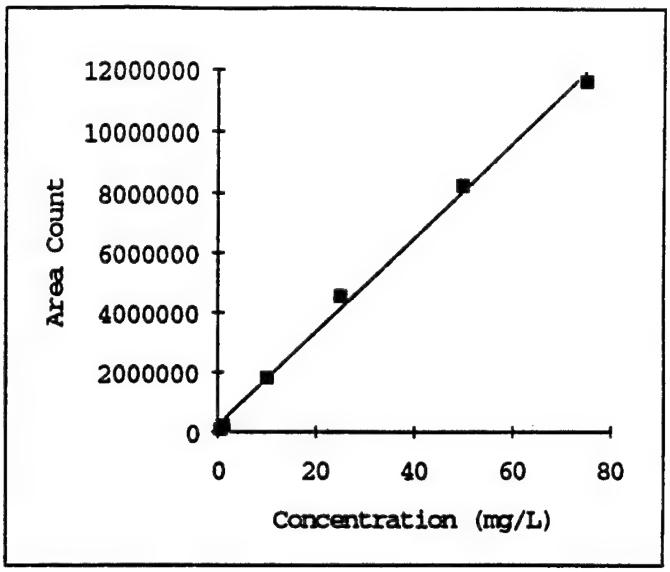
	df	Sum of Squares	Mean Square	F	Significance F	F[0.05(1,4)]
Regression	1	1.48398E+14	1.48398E+14	3841.0183	4.0598E-07	
Residual	4	1.5454E+11	38635081550			
Total	5	1.48553E+14	2.97105E+13			
LOF	-1	-1.48398E+14	1.48398E+14	4.9947985	Significant Fit	7.71

	Coefficients	Standard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	-117994.74	108857.9764	-1.08393286	0.3278724	-420233.5595	184244.084
Concentration	409785.954	6612.015787	61.97594911	2.07E-08	391428.0171	428143.891

Regression Analysis of Diethanolamine Analytical

27-Aug-93

Concentration	Area Count	Predicted
75	11620246	11921923.45
50	8261499	8027968.971
25	4577131	4134014.49
10	1812825	1797641.801
1	225096	395818.1876
0.5	98509	317939.0979



Regression Statistics

Multiple R	0.99808638
R Square	0.99617643
Adjusted R Square	0.99522053
Standard Error	323814.748
Observations	6

Analysis of Variance

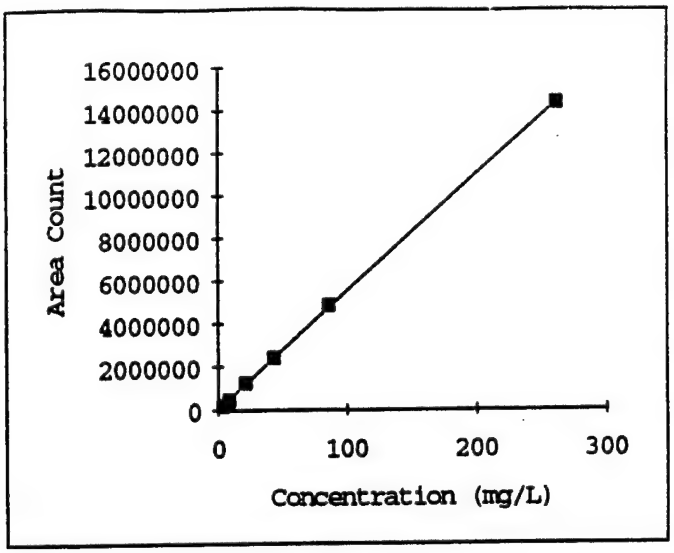
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,4)]*
Regression	1	1.09275E+14	1.09275E+14	1042.1421	5.48939E-06	
Residual	4	4.19424E+11	1.04856E+11			
Total	5	1.09694E+14	2.19389E+13			
LOF	-1	-1.09275E+14	1.09275E+14	4.9808821	Significant Fit	7.71

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	240060.008	185316.4911	1.295405535	0.2517608	-274462.1219	754582.139
Concentration	155758.179	4824.889703	32.28222589	5.358E-07	142362.1101	169154.248

Regression Analysis of HAN in LGP

26-Aug-93

Concentration	Area Count	Predicted
261.7	14407176	14446296.69
86.4	4880771	4784114.087
43.2	2450976	2403017.177
21.6	1245084	1212468.721
8.7	434240	501446.7274
4.3	188024	258927.5976



Regression Statistics

Multiple R	0.99991872
R Square	0.99983744
Adjusted R Square	0.99979681
Standard Error	77105.2148
Observations	6

Analysis of Variance

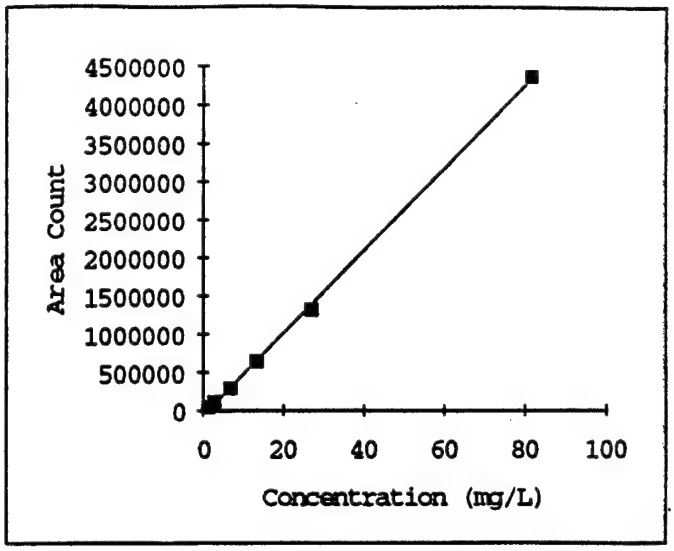
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,4)]*
Regression	1	1.4627E+14	1.4627E+14	24603.032	9.9096E-09	
Residual	4	23780856599	5945214150			
Total	5	1.46294E+14	2.92588E+13			
LOF	-1	-1.4627E+14	1.4627E+14	4.9991872	Significant Fit	7.71

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	21920.2662	40162.68792	0.545786833	0.6086702	-89589.46303	133429.995
Concentration	55117.984	351.3977753	156.8535372	1.998E-10	54142.34539	56093.6227

Regression Analysis of TEAN in LGP

26-Aug-93

Concentration	Area Count	Predicted
81.5	4364973	4340780.376
26.9	1324234	1387328.918
13.5	635884	662489.1824
6.7	288050	294660.063
2.7	103475	78289.9928
1.3	49493	2560.468227



Regression Statistics

Multiple R	0.99970184
R Square	0.99940376
Adjusted R Square	0.99925471
Standard Error	45151.7648
Observations	6

Analysis of Variance

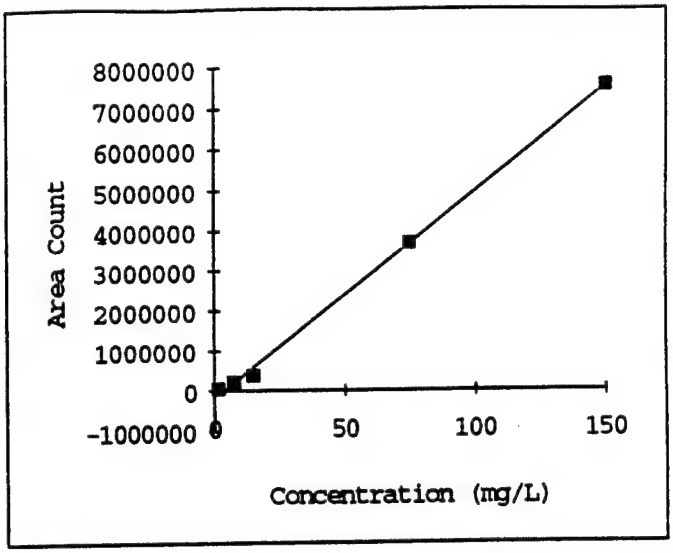
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,4)]*
Regression	1	1.36689E+13	1.36689E+13	6704.7585	1.33338E-07	
Residual	4	8154727470	2038681868			
Total	5	1.3677E+13	2.7354E+12			
LOF	-1	-1.36689E+13	1.36689E+13	4.9970188	Significant Fit	7.71

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-67759.805	23514.37067	-2.88163377	0.0345222	-133046.2992	-2473.31
Concentration	54092.5176	660.6107306	81.88258992	5.148E-09	52258.36432	55926.6708

Regression Analysis of HAN in HAN, TEAN, EA, DEA Mix

30-Aug-93

Concentration	Area Count	Predicted
150	7572456	7560796.382
75	3688981	3678599.988
15	384303	572842.8734
7.5	193095	184623.2341
1.5	32075	-125952.4774



Regression Statistics

Multiple R	0.99929159
R Square	0.99858367
Adjusted R Square	0.99811156
Standard Error	142402.54
Observations	5

Analysis of Variance

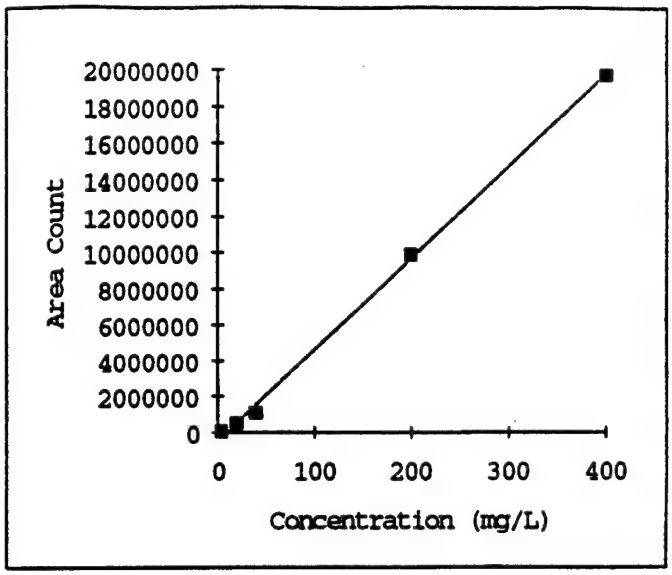
	df	Sum of Squares	Mean Square	F	Significance F	F[0.05(1,3)]
Regression	1	4.28921E+13	4.28921E+13	2115.1551	2.26318E-05	
Residual	3	60835450408	20278483469			
Total	4	4.2953E+13	1.07382E+13			
LOF	-1	-4.28921E+13	4.28921E+13	3.9943347	Significant Fit	10.1

	Coefficients	Standard Error	t Statistic	P-value	Lower 95%	Upper 95%
Intercept	-203596.41	84836.79998	-2.39985956	0.0743677	-473585.2192	66392.4087
Concentration	51762.6186	1125.499041	45.99081536	1.337E-06	48180.77495	55344.4622

Regression Analysis of TEAN in HAN, TEAN, EA, DEA Mix

30-Aug-93

Concentration	Area Count	Predicted
400	19725758	19792399.58
200	9882732	9657973.397
40	1080912	1550432.452
20	489497	536989.8341
4	85132	-273764.2603



Regression Statistics

Multiple R	0.99930542
R Square	0.99861132
Adjusted R Square	0.99814842
Standard Error	368089.246
Observations	5

Analysis of Variance

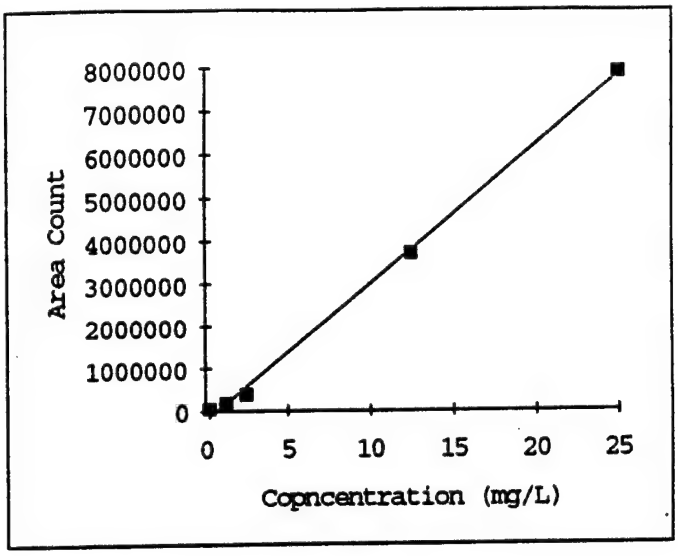
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	2.92295E+14	2.92295E+14	2157.3209	2.19722E-05	
Residual	3	4.06469E+11	1.3549E+11			
Total	4	2.92701E+14	7.31753E+13			
LOF	-1	-2.92295E+14	2.92295E+14	3.9944453	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-476452.78	219290.4263	-2.17270216	0.0955209	-1174333.446	221427.878
Concentration	50672.1309	1090.967442	46.44696894	1.285E-06	47200.18234	54144.0795
Concentration	50672.2124	1091.093465	46.44167899	1.286E-06	47199.8628	54144.562

Regression Analysis of EA in HAN, TEAN, EA, DEA Mix

30-Aug-93

Concentration	Area Count	Predicted
25	7909352	7847675.188
12.5	3710447	3800551.544
2.5	369353	562852.6293
1.25	170794	158140.265
0.25	43644	-165629.6265



Regression Statistics

Multiple R	0.99900051
R Square	0.99800201
Adjusted R Square	0.99733602
Standard Error	176371.325
Observations	5

Analysis of Variance

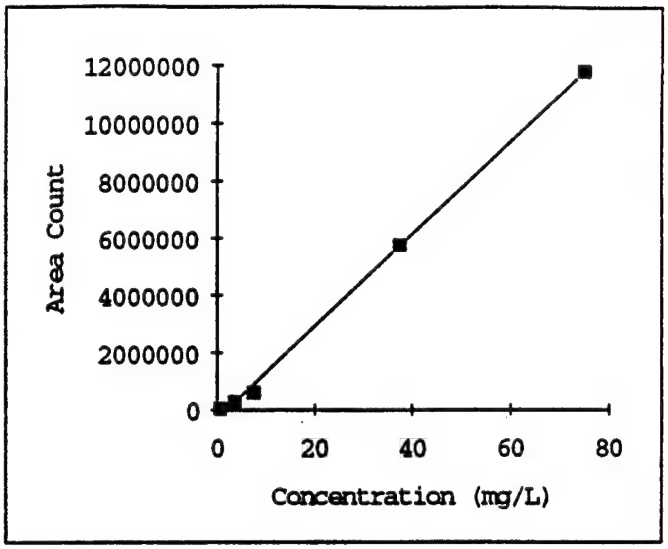
	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	4.66139E+13	4.66139E+13	1498.5101	3.79262E-05	
Residual	3	93320532366	31106844122			
Total	4	4.67072E+13	1.16768E+13			
LOF	-1	-4.66139E+13	4.66139E+13	3.992008	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-246572.1	105073.8193	-2.34665591	0.0788028	-580964.2011	87820.0023
Concentration	323769.891	8363.857398	38.71059442	2.66E-06	297152.3394	350387.444

Regression Analysis of DEA in HAN, TEAN, EA, DEA Mix

30-Aug-93

Concentration	Area Count	Predicted
75	11809793	11792811.19
37.5	5749355	5732427.515
7.5	609032	884120.5749
3.75	269370	278082.2074
0.75	43143	-206748.4866



Regression Statistics

Multiple R	0.99933685
R Square	0.99867414
Adjusted R Square	0.99823219
Standard Error	215073.866
Observations	5

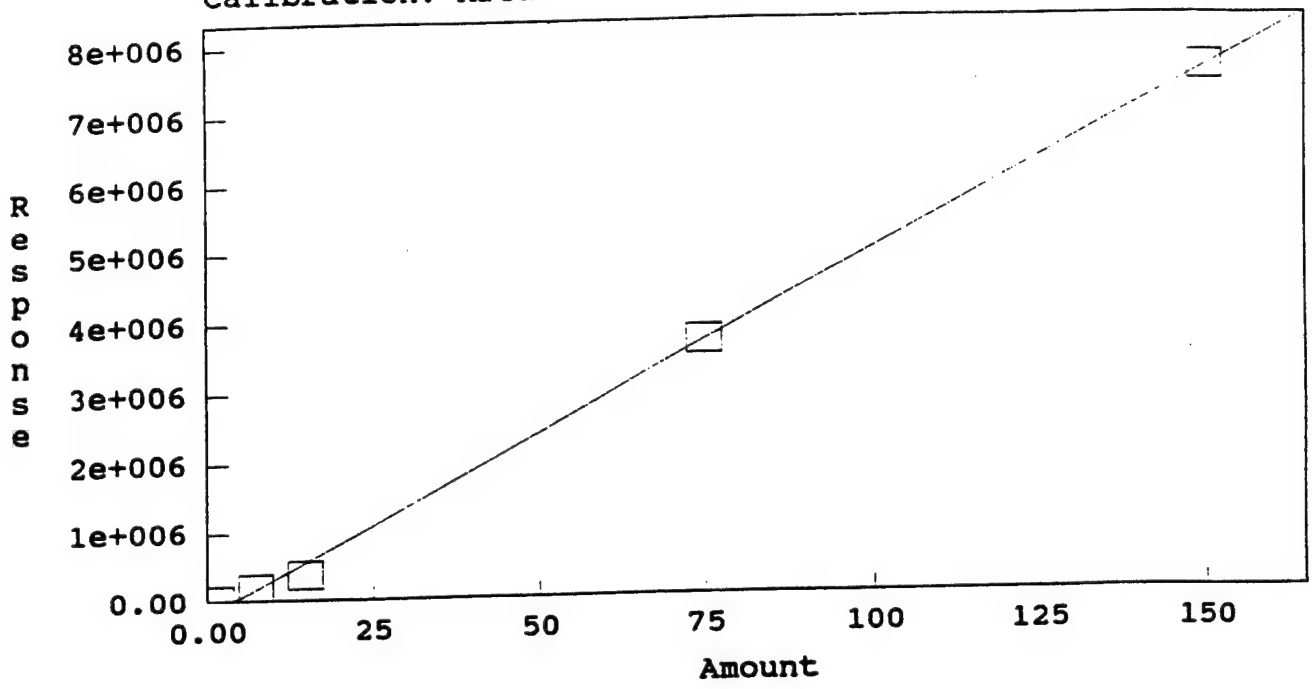
Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*	*F[0.05(1,3)]*
Regression	1	1.04526E+14	1.04526E+14	2259.6837	2.04978E-05	
Residual	3	1.3877E+11	46256767767			
Total	4	1.04664E+14	2.61661E+13			
LOF	-1	-1.04526E+14	1.04526E+14	3.9946966	Significant Fit	10.1

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-327956.16	128130.9905	-2.55953816	0.0626687	-735726.5401	79814.2198
Concentration	161610.231	3399.734714	47.53613	1.172E-06	150790.748	172429.715

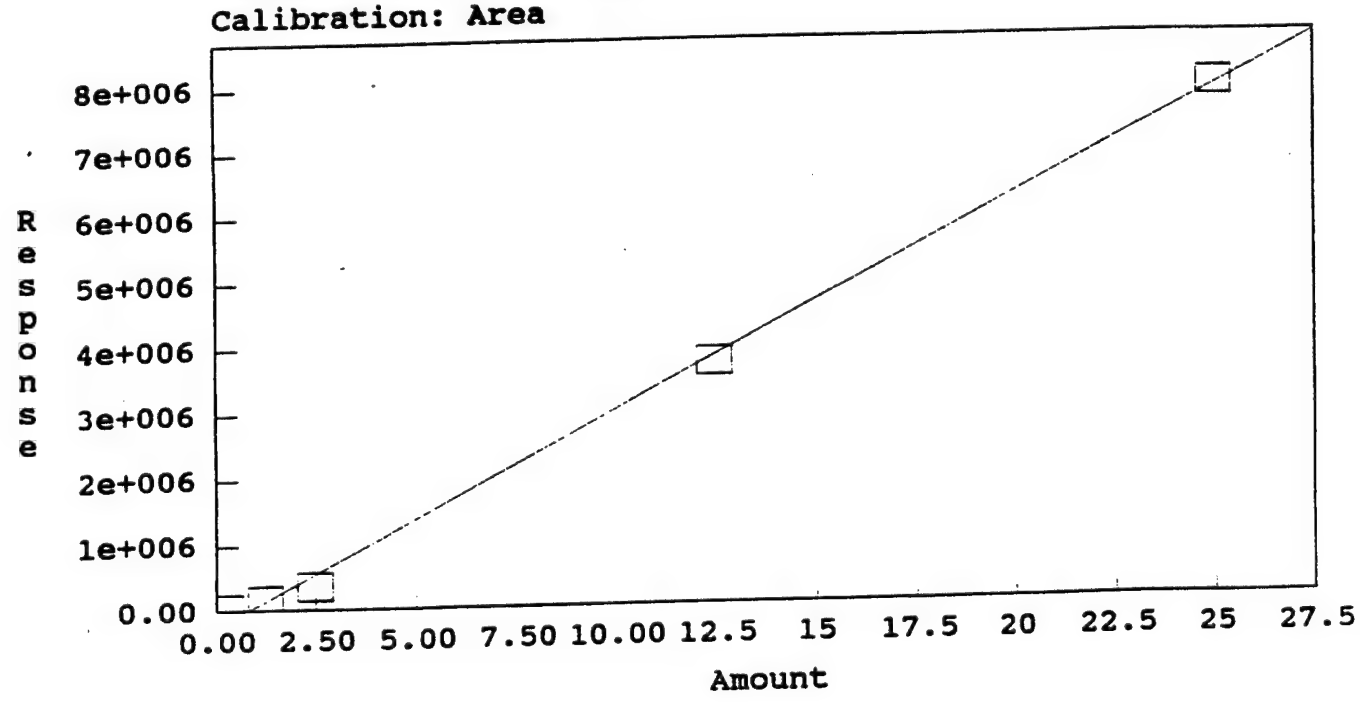
-Method Updated: 15:06 on Mon, 30 Aug 1993

Component: HAN
Fit Type: Linear
r^2 = 0.998584
Amt = Resp * 1.929e-005 + 3.998
Resp = Amt * 5.184e+004 + -2.073e+005
Standardization: External
Calibration: Area



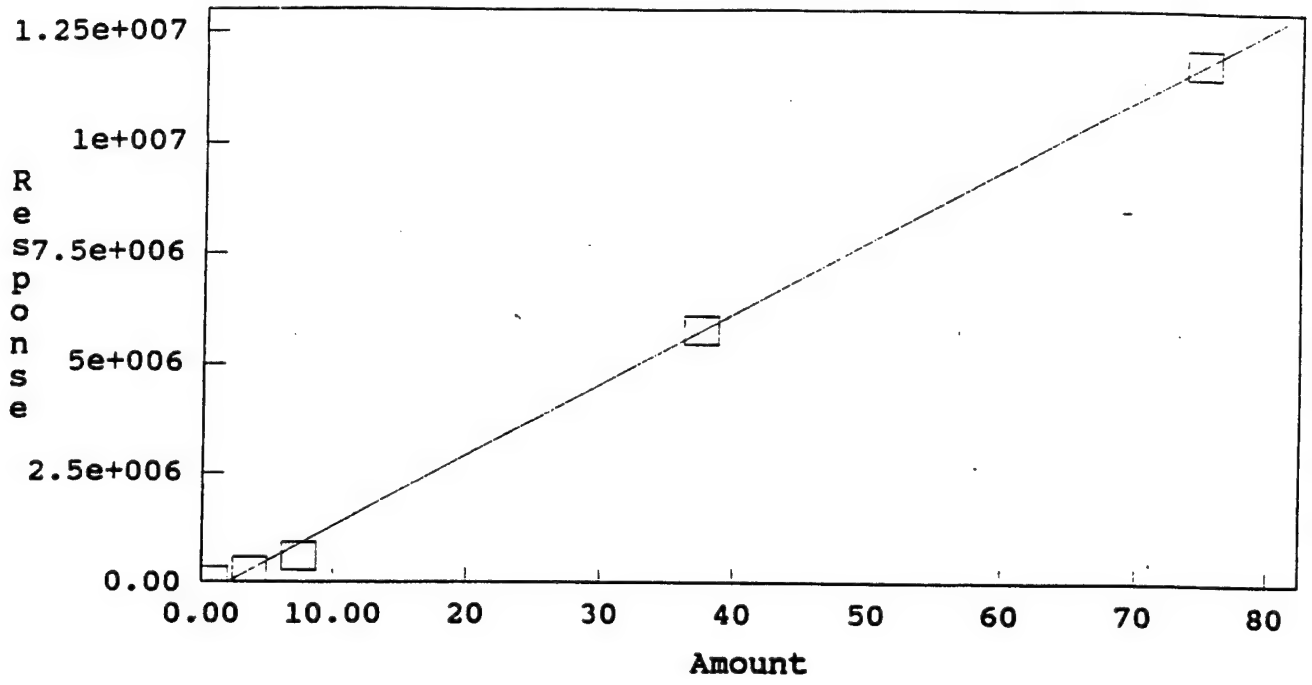
Method: C:\DX\METHOD\MIX830.MET

Component: Ethanolamine
Fit Type: Linear
r^2 = 0.998002
Amt = Resp * 3.082e-006 + 0.7766
Resp = Amt * 3.244e+005 + -2.52e+005
Standardization: External
Calibration: Area



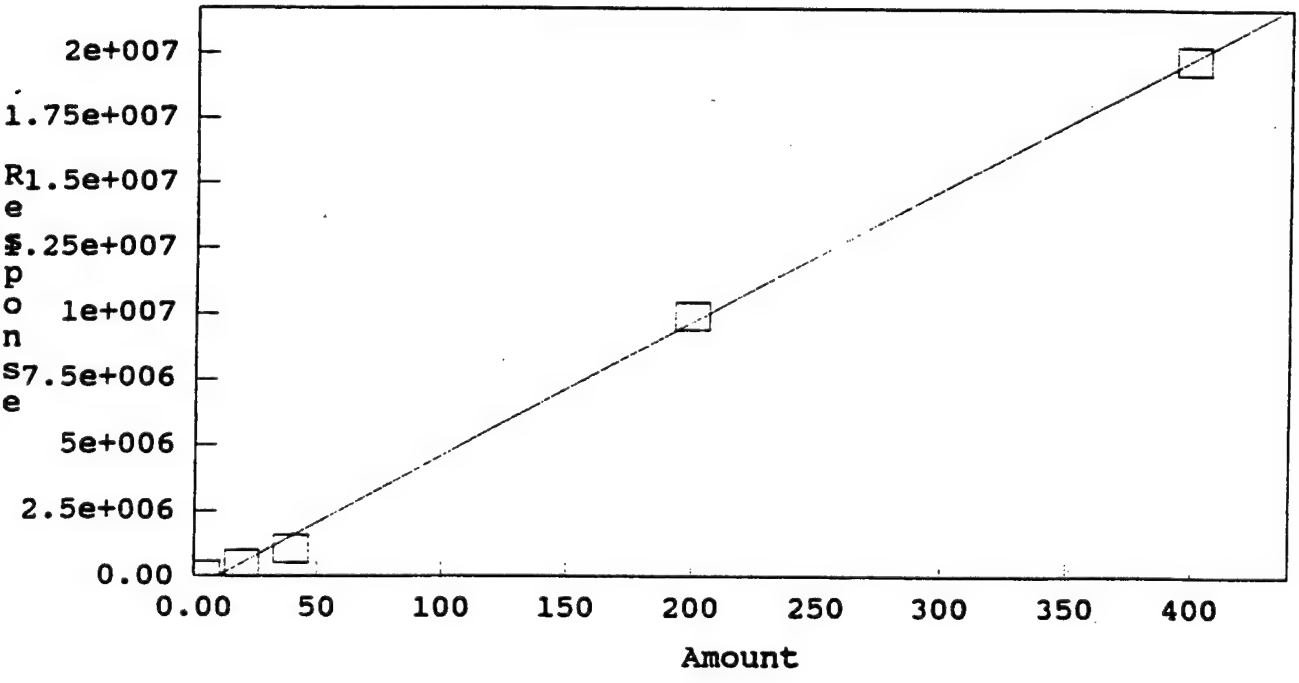
Method: C:\DX\METHOD\MIX830.MET

Component: Diethanolamine
Fit Type: Linear
r^2 = 0.998674
Amt = Resp * 6.18e-006 + 2.06
Resp = Amt * 1.618e+005 + -3.333e+005
Standardization: External
Calibration: Area



Method: C:\DX\METHOD\MIX830.MET

Component: TEAN
Fit Type: Linear
r^2 = 0.998611
Amt = Resp * 1.971e-005 + 9.574
Resp = Amt * 5.074e+004 + -4.858e+005
Standardization: External
Calibration: Area



DIONEX METHOD PARAMETERS - MIX830.MET

Method Comment: WES Verification
Column ID: Waters IC-Pak Cation M/D
Analyst ID: J.Rightmyer

System Parameters

System Name: pah/anions	
Number of Detectors	1
Run Time (minutes)	12.00
Sampling Rate (seconds)	0.20
Detector 1 Type	PAD
Detector 1 real time plot scale maximum (nA)	2000.0
minimum	900.0
Detector 1 Output Equivalent to 1 Volt (in nA)	10000.00
Detector 1 ACI Analog Input Connection	DET2
Save Data File	Yes
Data File Name: C:\DX\DATA\WES830A1.D06	

-- DETECTOR 1 PARAMETERS --

Report Options

Create ASCII Report File	No
Print Report	Yes
Print All Components	Yes
Print Components Found	No
Print Missing Components	No
Print All Peaks	No
Print Unknown Peaks	Yes
Print Chromatogram	Yes
Autoscale Chromatogram Maximum	Yes
Autoscale Maximum Value Delay (minutes)	48.0
Autoscale Chromatogram Minimum	No
Fill Peaks with Color	Yes
Draw Grid Lines on Chromatogram	No
Show Component Fraction Numbers	No
Label with Peak Number	Yes
Label with Retention Times on Chromatogram	No
Label with Component Name	No
Format File Name: C:\DX\METHOD\default.prf	

Integration Parameters

Starting Peak Width (seconds)	10.0
Peak Threshold	0.500
Peak Area Reject	10000
Area Reject for Reference Peaks	10000

Calibration Parameters

Number Of Levels for Calibration	5
Force Calibration Curve Through Origin	No
Calibration Fit Type	Linear
Replace Or Average Calibrations	Replace
External or Internal Calibration	External
Calculate Unknowns by Area or Height	Area
Default Sample Volume	1.0
Default Dilution Factor	1.0
Default Response Factor for Unknown Peaks	0.0
Calibration Standard Volume	1.0
Internal Standard Amount in Samples	1.0
Amount Units	

Component Table -- Last Modified: 15:06 on Mon, 30 Aug 1993

Component # 1 HAN
Reference Comp. none
Amount = K0 + K1*Area
K0 = 3.99823E+000
K1 = 1.92916E-005
Retention Time 3.53
Window Size 0.50 min.

Level	Amount	Area	Height
1	1.50000E+002	7572456	717680
2	7.50000E+001	3688981	374616
3	1.50000E+001	384303	40726
4	7.50000E+000	193095	20401
5	1.50000E+000	32075	2375

Component # 2 Ethanolamine
Reference Comp. none
Amount = K0 + K1*Area
K0 = 7.76628E-001
K1 = 3.08244E-006
Retention Time 4.53
Window Size 0.50 min.

Level	Amount	Area	Height
1	2.50000E+001	7909352	713686
2	1.25000E+001	3710447	341965
3	2.50000E+000	369353	34146
4	1.25000E+000	170794	16194
5	2.50000E-001	43644	3007

Component # 3 Diethanolamine
Reference Comp. none
Amount = K0 + K1*Area
K0 = 2.05963E+000
K1 = 6.17952E-006
Retention Time 5.27
Window Size 0.50 min.

Level	Amount	Area	Height
1	7.50000E+001	11809793	919983
2	3.75000E+001	5749355	455549
3	7.50000E+000	609032	47225
4	3.75000E+000	269370	22728
5	7.50000E-001	43143	3045

Component # 4 TEAN
Reference Comp. none
Amount = K0 + K1*Area
K0 = 9.57402E+000
K1 = 1.97073E-005
Retention Time 6.68
Window Size 0.50 min.

Level	Amount	Area	Height
1	4.00000E+002	19725758	1127049
2	2.00000E+002	9882732	610328
3	4.00000E+001	1080912	65975
4	2.00000E+001	489497	31945
5	4.00000E+000	85132	4211

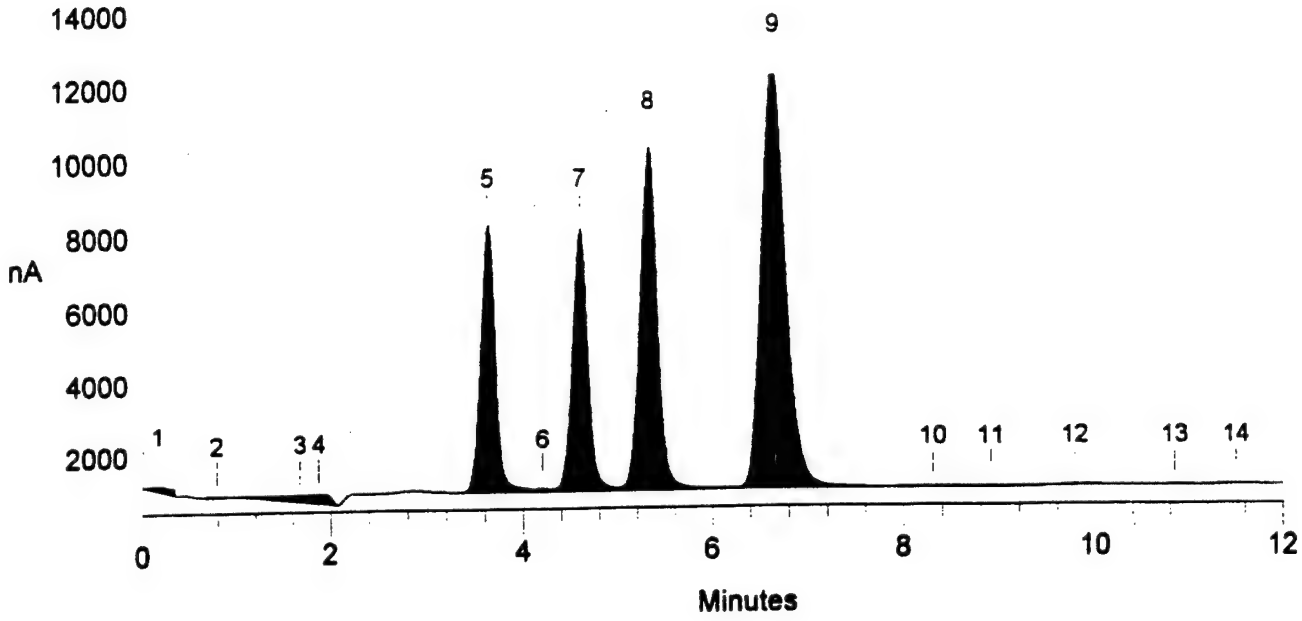
Timed Events File: C:\DX\METHOD\WES.TE

Step	Time	Description
Init		ACI Autosmp OFF
Init		ACI RLY 2 OFF
Init		ACI RLY 3 OFF
Init		ACI RLY 4 OFF
Init		ACI TTL 1 OFF
Init		ACI TTL 2 OFF
Init		ACI TTL 3 OFF
Init		ACI TTL 4 OFF
Init		ACI AC2 OFF
Init		ACI AC 2 OFF
Init		PAD Cell ON
Init		PAD AutoOffset OFF
Init		PAD Recorder Mark OFF
Init		PAD Recorder Range = 3000.0 nA
Init		GPM Start
Init		GPM Hold Gradient Clock
Init		GPM Reset OFF
1	0.3	ACI Autosmp ON
1	0.3	Start Sampling
1	0.3	GPM Run Gradient Clock
2	0.6	PAD AutoOffset ON

Lo Pressure Limit = 0
Hi Pressure Limit = 3000
Eluent 1 -
Eluent 2 -
Eluent 3 - 5% MeOH,0.1mM EDTA, 2mM HNO3
Eluent 4 -
V5 Off - Off
V5 On - On
V6 Off - Off
V6 On - On

Time	Flow	%1	%2	%3	%4	V5	V6	Comment
0.0	0.9	0	100	0	0	1	1	

File: WES830A1.D02 Sample: AUTOCAL1



Data Reprocessed On 08/30/1993 14:25:51

Sample Name: AUTOCAL1 Date: 08/30/1993 14:09:13
Data File : C:\DX\DATA\WES830A1.D02
Method : C:\DX\METHOD\mix830.met
ACI Address: 1 System: 1 Inject#: 2 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3600	5Hz	0.00	12.00	10000

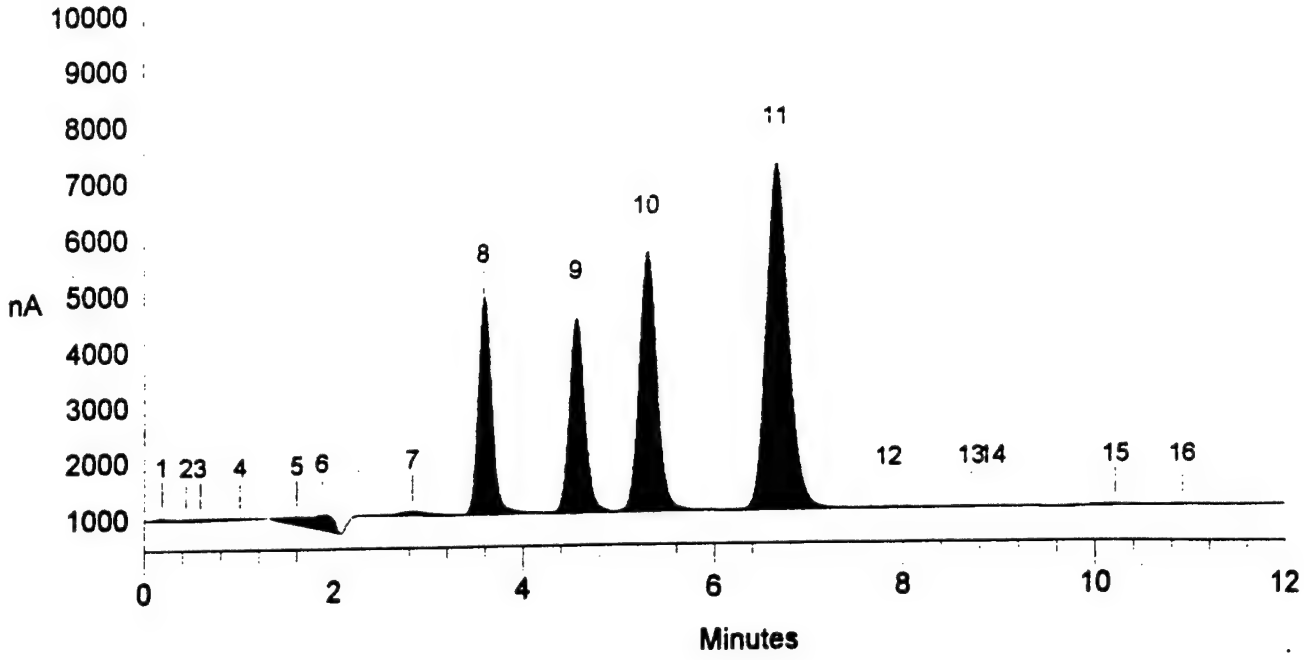
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
5	3.62	HAN	150.000	717680	7572456	3	0.00
7	4.58	Ethanolamine	25.000	713686	7909352	2	0.00
8	5.30	Diethanolamine	75.000	919983	11809793	2	0.00
9	6.62	TEAN	400.000	1127049	19725758	2	0.00
		Totals	650.000	3478398	47017359		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.15		0.000	11612	208376	1	
3	1.67		0.000	18937	454074	2	
4	1.87		0.000	25631	350268	2	
6	4.20		0.000	5636	52673	4	
10	8.30		0.000	2118	76394	2	
11	8.90		0.000	1804	42499	2	
12	9.78		0.000	1954	84200	2	
13	10.85		0.000	1096	31431	2	
		Totals	0.000	68788	1299915		

File: WES830A1.D03 Sample: AUTOCAL2



Sample Name: AUTOCAL2 Date: 08/30/1993 14:21:54
Data File : C:\DX\DATA\WES830A1.D03
Method : C:\DX\METHOD\MIX830.MET
ACI Address: 1 System: 1 Inject#: 3 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3600	5Hz	0.00	12.00	10000

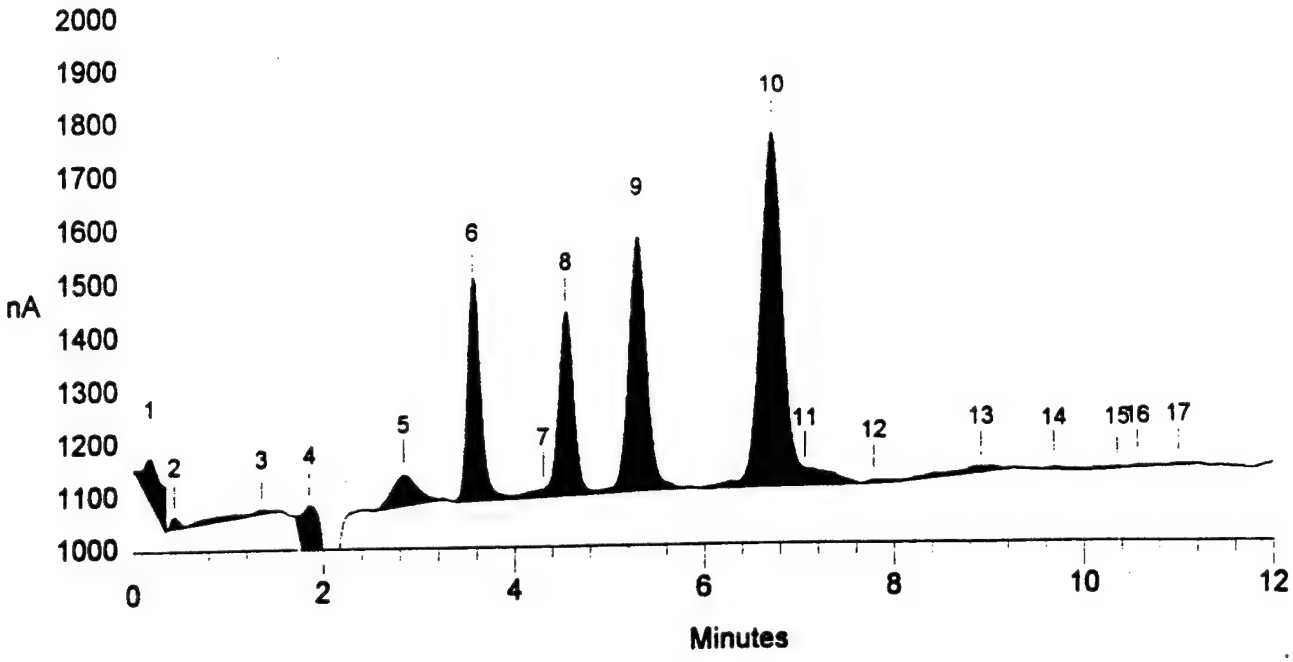
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
8	3.58	HAN	75.000	374616	3688981	1	-1.01
9	4.55	Ethanolamine	12.500	341965	3710447	1	-0.66
10	5.28	Diethanolamine	37.500	455549	5749355	1	-0.31
11	6.63	TEAN	200.000	610328	9882732	1	0.20
		Totals	325.000	1782457	23031516		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.18		0.000	3439	32541	1	
2	0.43		0.000	1986	13259	2	
3	0.58		0.000	1832	26209	2	
5	1.60		0.000	13494	267360	2	
6	1.87		0.000	27201	375984	2	
7	2.83		0.000	6557	127264	1	
12	7.85		0.000	555	13519	1	
15	10.22		0.000	544	19890	1	
16	10.92		0.000	895	14134	1	
		Totals	0.000	56503	890160		

File: WES830A1.D04 Sample: AUTOCAL3



Data Reprocessed On 08/30/1993 14:58:38

Sample Name: AUTOCAL3
Data File : C:\DX\DATA\WES830A1.D04 Date: 08/30/1993 14:34:38
Method : C:\DX\METHOD\MIX830.MET
ACI Address: 1 System: 1 Inject#: 4 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3600	5Hz	0.00	12.00	10000

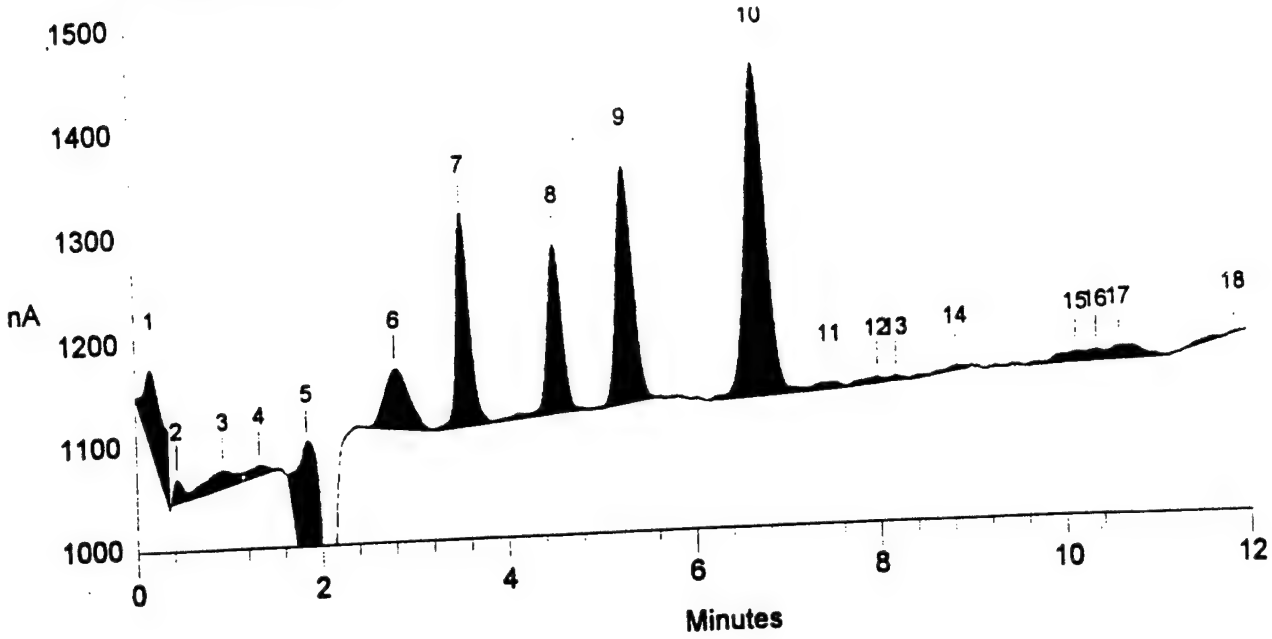
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
6	3.55	HAN	15.000	40726	384303	2	0.00
8	4.53	Ethanolamine	2.500	34146	369353	2	0.00
9	5.28	Diethanolamine	7.500	47225	609032	2	0.00
10	6.70	TEAN	40.000	65975	1080912	3	0.00
		Totals	65.000	188072	2443601		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	7778	114794	1	
2	0.43		0.000	2045	12699	2	
3	1.35		0.000	574	25524	2	
4	1.85		0.000	14855	247059	1	
5	2.83		0.000	5698	101623	1	
7	4.30		0.000	1541	17836	2	
11	7.06		0.000	848	45422	4	
13	8.92		0.000	1006	34546	1	
		Totals	0.000	34346	599503		

File: WES830A1.D05 Sample: AUTOCAL4



Data Reprocessed On 08/30/1993 15:03:33

Sample Name: AUTOCAL4 Date: 08/30/1993 14:47:18
Data File : C:\DX\DATA\WES830A1.D05
Method : C:\DX\METHOD\MIX830.MET
ACI Address: 1 System: 1 Inject#: 5 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3600	5Hz	0.00	12.00	10000

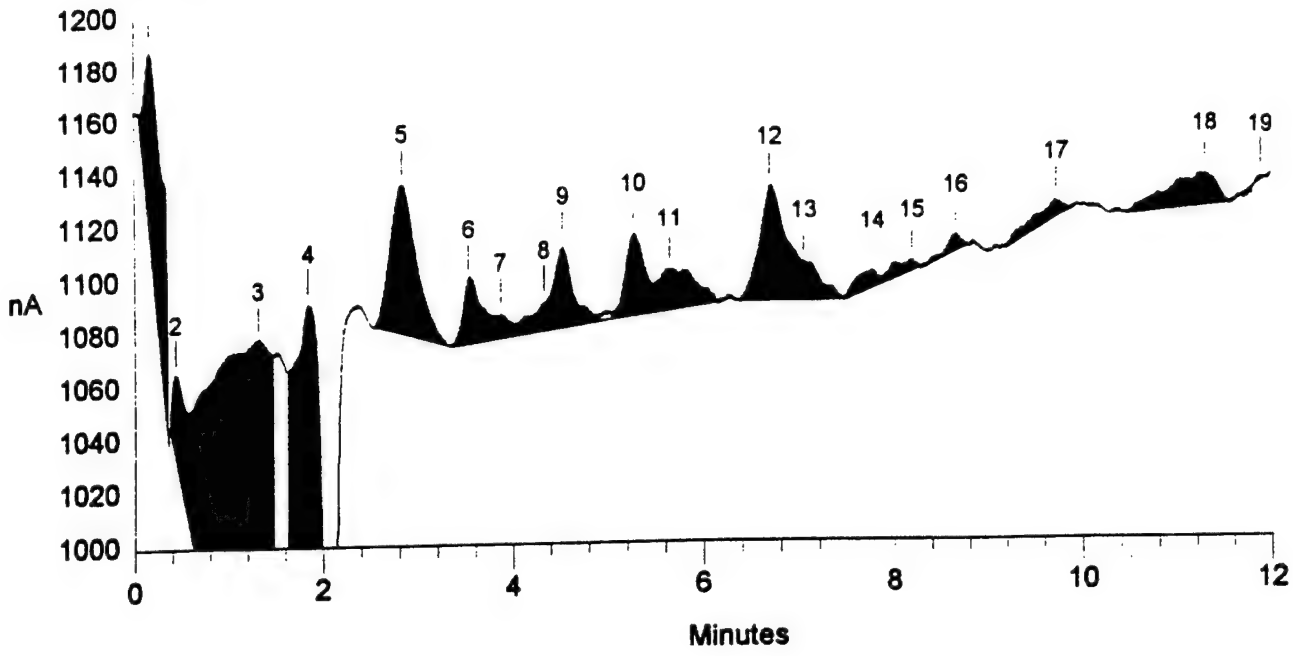
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
7	3.55	HAN	7.500	20401	193095	1	0.00
8	4.53	Ethanolamine	1.250	16194	170794	1	0.07
9	5.28	Diethanolamine	3.750	22728	269370	1	0.06
10	6.70	TEAN	20.000	31945	489497	3	0.00
		Totals	32.500	91268	1122756		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	7954	111459	1	
2	0.43		0.000	2233	15370	2	
3	0.93		0.000	1588	38016	2	
4	1.33		0.000	916	10982	2	
5	1.85		0.000	18696	318771	1	
6	2.82		0.000	5744	104895	1	
15	10.13		0.000	954	19355	2	
16	10.35		0.000	1065	11015	2	
17	10.60		0.000	1231	29819	2	
		Totals	0.000	40380	659683		

File: WES830A1.D06 Sample: AUTOCAL5



Sample Name: AUTOCAL5 Date: 08/30/1993 15:00:11
Data File : C:\DX\DATA\WES830A1.D06
Method : C:\DX\METHOD\MIX830.MET
ACI Address: 1 System: 1 Inject#: 6 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3600	5Hz	0.00	12.00	10000

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
6	3.53	HAN	1.500	2375	32075	2	-0.47
9	4.53	Ethanolamine	0.250	3007	43644	2	0.07
10	5.27	Diethanolamine	0.750	3045	43143	2	-0.25
12	6.68	TEAN	4.000	4211	85132	2	-0.25
		Totals	6.500	12638	203993		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration	Height	Area	Bl. Code	%Delta
1	0.17		0.000	7176	106460	1	
2	0.43		0.000	3170	36413	2	
3	1.32		0.000	20207	749901	2	
4	1.85		0.000	31106	640078	2	
5	2.83		0.000	5571	107126	1	
7	3.87		0.000	906	10993	2	
8	4.34		0.000	1081	12259	2	
11	5.65		0.000	1462	38240	2	
13	7.04		0.000	1436	17708	2	
14	7.77		0.000	567	10982	2	
17	9.72		0.000	539	14828	1	
18	11.28		0.000	1166	42596	1	
		Totals	0.000	74386	1787585		

Appendix B

Selected Chromatograms Developed During WES Method Validation

Sample Name: GRDWATER BLANK A

Untreated groundwater chromatogram showing background peaks equivalent to 3 mg/L TEAN.

Data Reprocessed On 11/18/1993 22:34:08

Sample Name: GRDWATER BLANK A Date: 10/04/1993 13:06:08
Data File : B:\AQ102001.D17
Method : C:\DX\METHOD\aq102.met
ACI Address: 1 System: 1 Inject#: 17 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

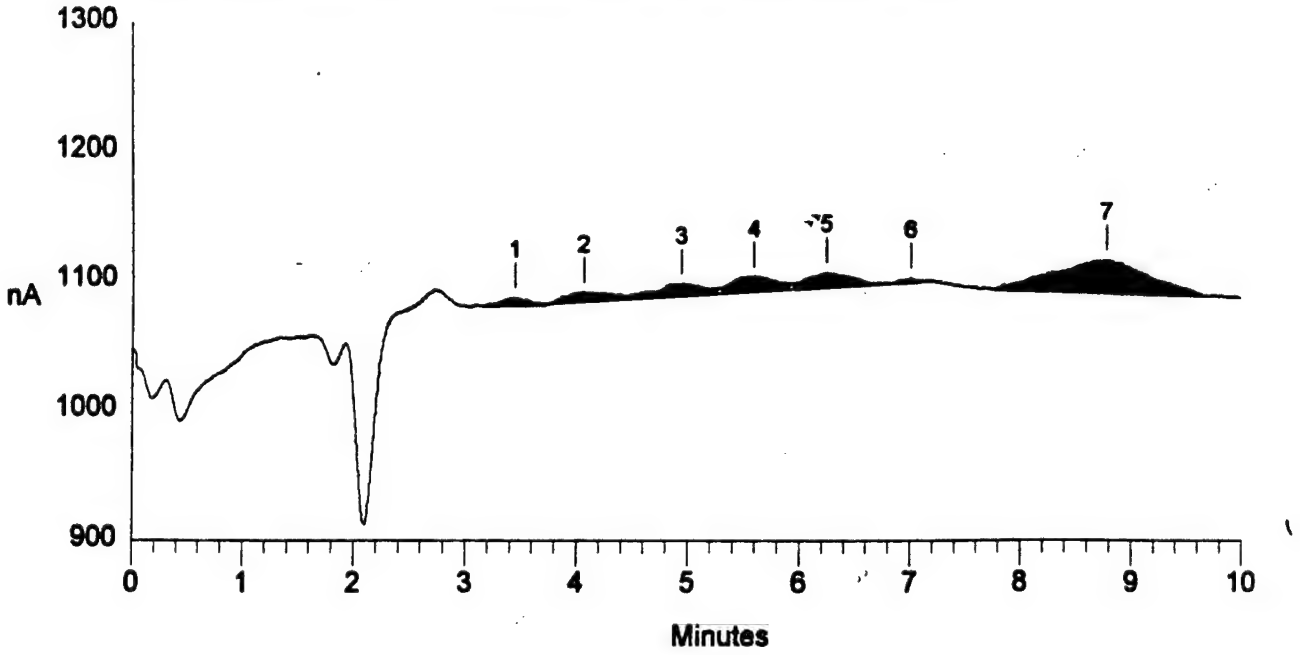
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
0	0.00	HAN	0.000	0	0	0	0.00
5	6.25	TEAN	3.076	1132	28964	2	0.00
		Totals	3.076	1132	28964		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
2	4.07		0.000	692	19377	2	
3	4.95		0.000	853	27304	2	
4	5.60		0.000	1092	30201	2	
7	8.78		0.000	2544	150129	1	
		Totals	0.000	5181	227010		

File: AQ102001.D17 Sample: GRDWATER BLANK A



Sample Name: K. GRDWATER 53 B

Groundwater sample spiked with 32.3 mg of HAN/L and 10 mg of TEAN/L.

Data Reprocessed On 11/18/1993 22:20:48

Sample Name: K. GRDWATER 53 B Date: 10/04/1993 12:02:27
Data File : B:\AQ102001.D11
Method : C:\DX\METHOD\aq102.met
ACI Address: 1 System: 1 Inject#: 11 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

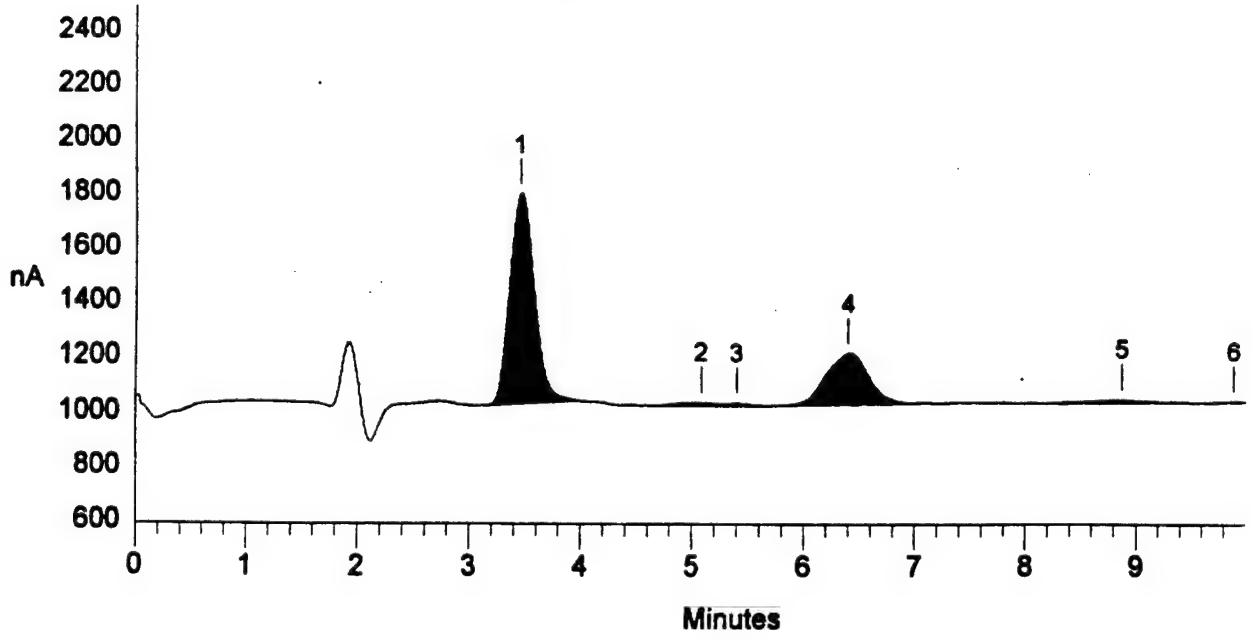
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	3.45	HAN	20.582	76476	1130657	1	-0.00
4	6.40	TEAN	14.439	18203	475017	1	0.00
		Totals	35.021	94679	1605674		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
2	5.08		0.000	929	22566	2	
3	5.40		0.000	849	10115	2	
5	8.87		0.000	1063	32787	1	
		Totals	0.000	2841	65468		

File: AQ102001.D11 Sample: K. GRDWATER 53 B



Sample Name: SEAWATER 10.5 B

Untreated seawater chromatogram showing background peaks equivalent to 4 mg/L TEAN.

Data Reprocessed On 11/18/1993 22:45:46

Sample Name: SEAWATER 10.5 B Date: 10/04/1993 14:10:05
Data File : B:\AQ102001.D23
Method : B:\AQ102.MET
ACI Address: 1 System: 1 Inject#: 23 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

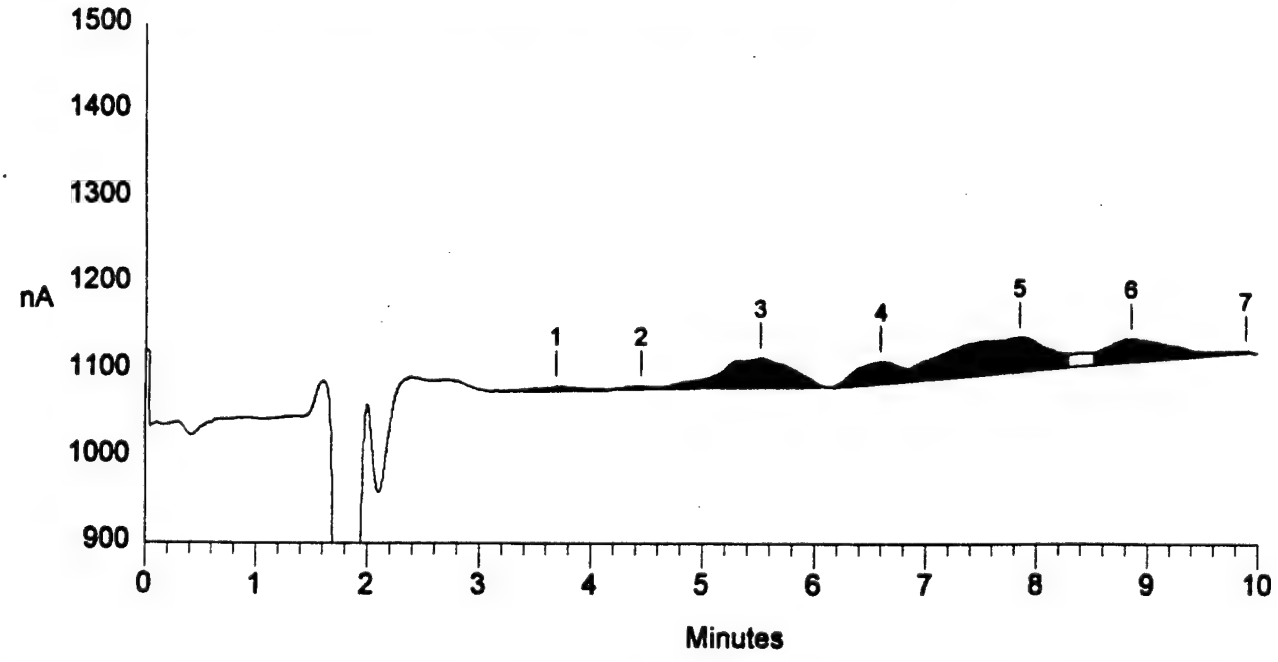
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
0	0.00	HAN	0.000	0	0	0	0.00
4	6.60	TEAN	4.128	2525	70265	2	-0.00
		Totals	4.128	2525	70265		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
3	5.52		0.000	3414	151558	2	
5	7.85		0.000	3895	257691	2	
6	8.85		0.000	2686	109542	3	
		Totals	0.000	9996	518791		

File: AQ102001.D23 Sample: SEAWATER 10.5 B



Sample Name: SEAWATER 527 A

Seawater chromatogram indicating HAN and TEAN peak distortion caused by seawater. Seawater was spiked with HAN and TEAN at concentrations of 312.5 and 100 mg/L, respectively. Chromatogram corresponds to data shown in Table 16.

Data Reprocessed On 11/18/1993 22:43:19

Sample Name: SEAWATER 527 A Date: 10/04/1993 13:59:23
Data File : B:\AQ102001.D22
Method : B:\AQ102.MET
ACI Address: 1 System: 1 Inject#: 22 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	10	3000	5Hz	0.00	10.00	10000

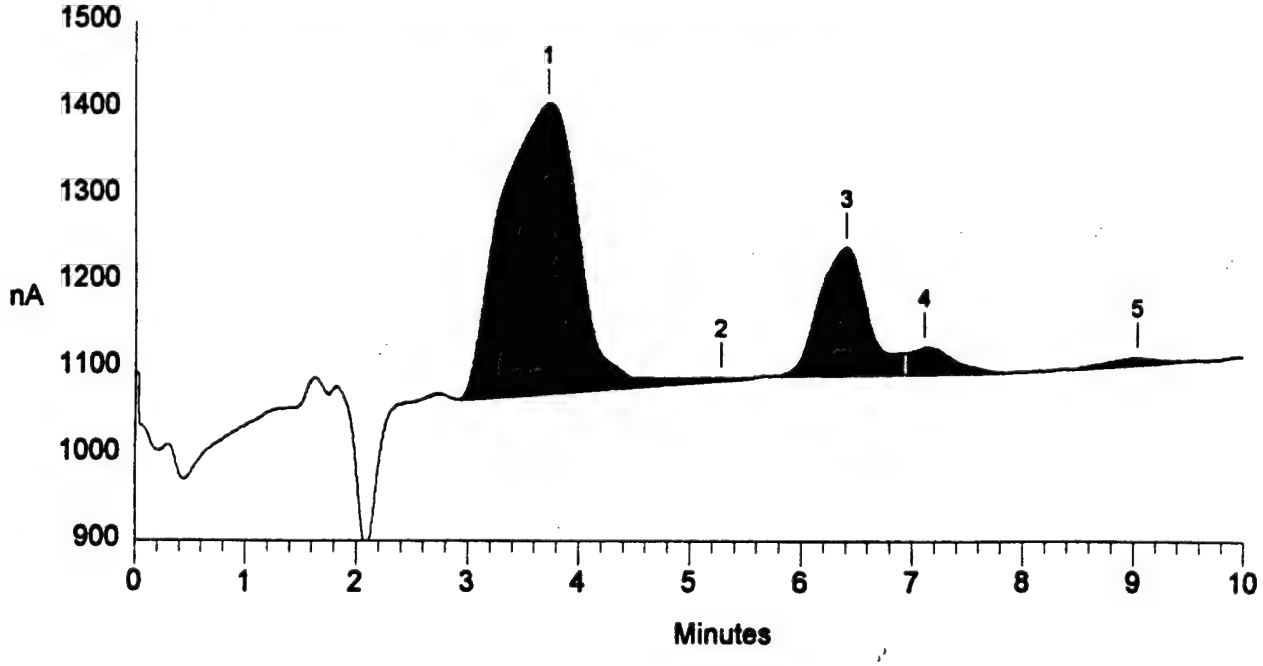
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	3.72	HAN	288.762	33523	1607766	3	0.00
3	6.40	TEAN	136.691	14774	444809	2	0.00
		Totals	425.453	48297	2052575		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
4	7.12		0.000	3133	82066	2	
5	9.03		0.000	845	25321	1	
		Totals	0.000	3978	107387		

File: AQ102001.D22 Sample: SEAWATER 527 A



Sample Name: SEAWATER 53 A

Seawater chromatogram indicating loss of HAN and TEAN peak distortion. Seawater was spiked with HAN and TEAN at concentrations of 32.3 and 10 mg/L, respectively. Chromatogram corresponds to data shown in Table 16.

Data Reprocessed On 11/18/1993 22:41:10

Sample Name: SEAWATER 53 A Date: 10/04/1993 13:48:42
Data File : B:\AQ102001.D21
Method : C:\DX\METHOD\aq102.met
ACI Address: 1 System: 1 Inject#: 21 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	1	3000	5Hz	0.00	10.00	10000

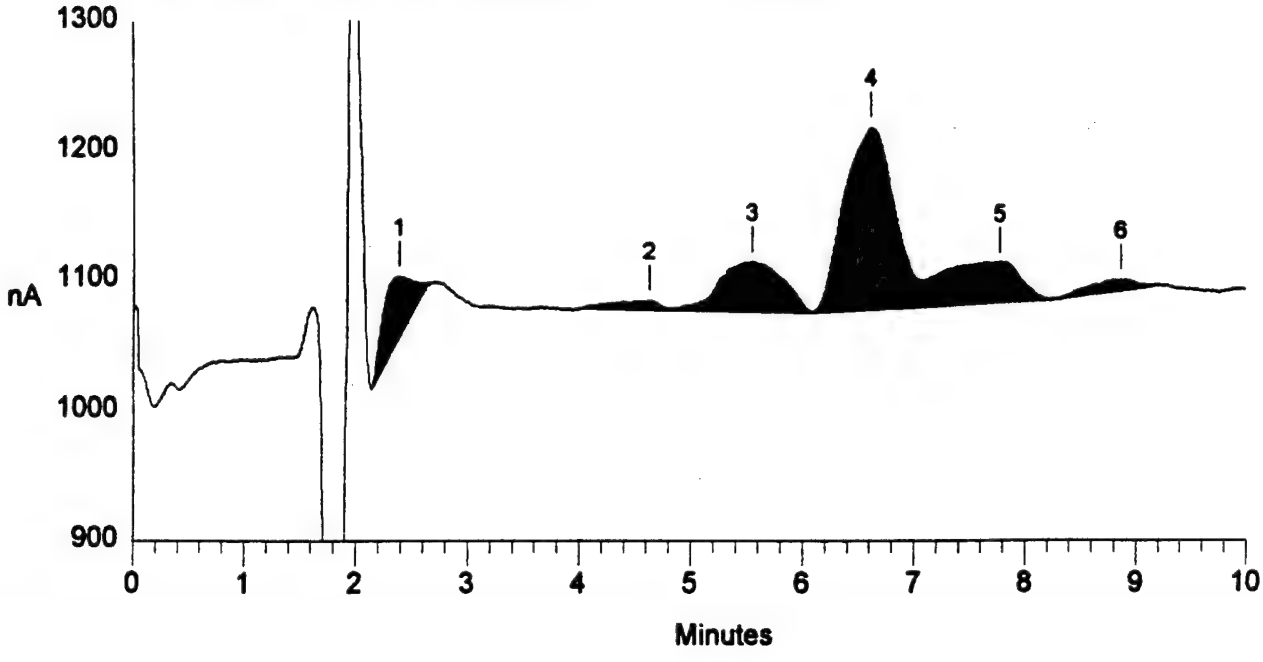
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
0	0.00	HAN	0.000	0	0	0	0.00
4	6.62	TEAN	13.441	14098	435849	2	0.00
		Totals	13.441	14098	435849		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	2.38		0.000	4601	87373	1	
2	4.63		0.000	674	19522	2	
3	5.55		0.000	3826	147423	2	
5	7.78		0.000	3031	154624	2	
6	8.87		0.000	825	23958	1	
		Totals	0.000	12957	432899		

File: AQ102001.D21 Sample: SEAWATER 53 A



Sample Name: ORGANIC 26C

Chromotagram of organic soil spiked with 26 mg/kg LGP.

Data Reprocessed On 11/11/1993 02:34:23

Sample Name: ORGANIC 26C Date: 11/11/1993 01:23:05
Data File : C:\DX\DATA\IMM11101.D10
Method : C:\DX\METHOD\IMM1110.MET
ACI Address: 1 System: 1 Inject#: 10 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	2.5	3000	5Hz	0.00	10.00	10000

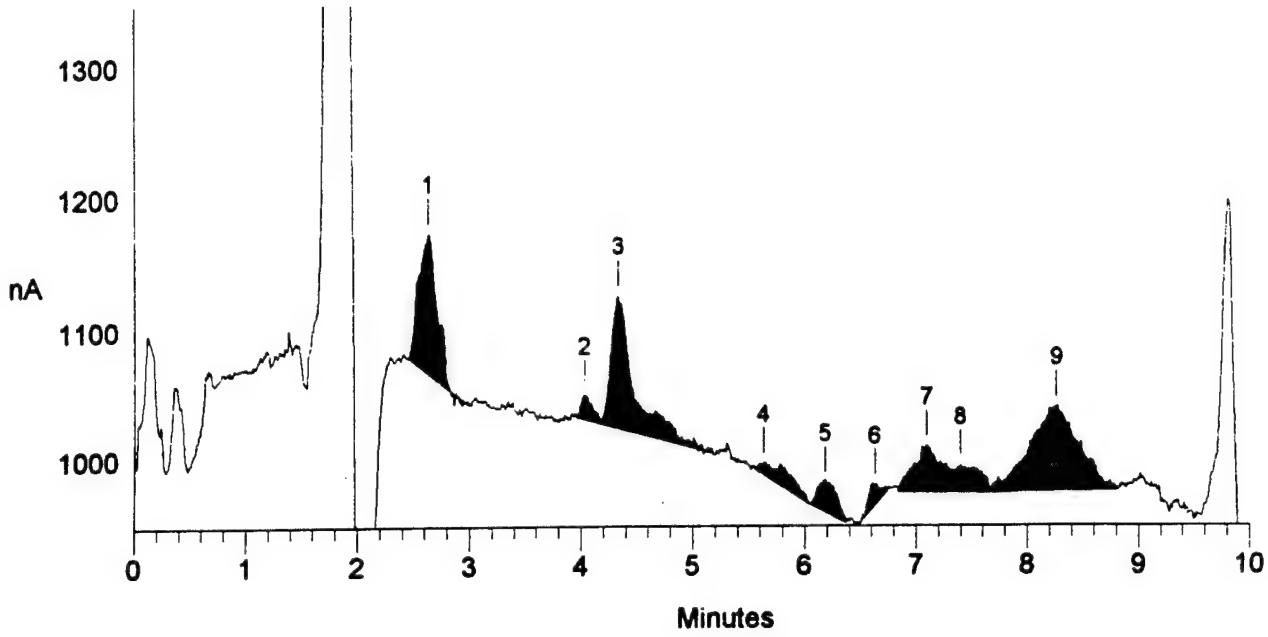
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
2	4.03	HAN	3.976	1788	13329	2	0.00
7	7.10	TEAN	10.259	3590	60573	2	0.00
		Totals	14.236	5378	73902		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	2.63		0.000	10561	117718	1	
3	4.33		0.000	10099	141688	2	
4	5.63		0.000	892	25005	1	
5	6.18		0.000	2256	27912	1	
6	6.63		0.000	1551	12022	1	
8	7.40		0.000	2080	33640	2	
9	8.27		0.000	6527	194299	1	
		Totals	0.000	33967	552285		

File: IMM11101.D10 Sample: ORGANIC 26C



Sample Name: CLAY 84A

Chromotagram of clay soil spiked with 84 mg/kg LGP.

Data Reprocessed On 11/11/1993 23:43:35

Sample Name: CLAY 84A Date: 11/11/1993 05:31:27
Data File : C:\DX\DATA\IMM11101.D33
Method : C:\DX\METHOD\imm1110.met
ACI Address: 1 System: 1 Inject#: 33 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	8	3000	5Hz	0.00	10.00	10000

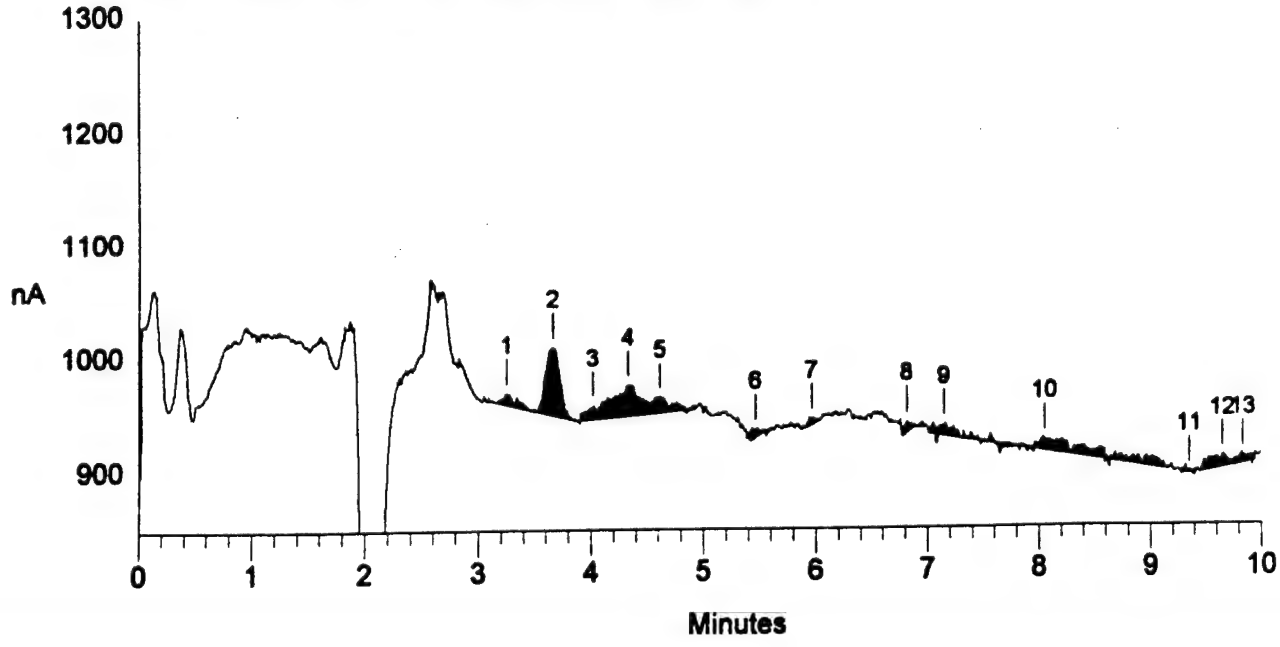
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
2	3.67	HAN	17.988	5857	50111	2	0.00
0	0.00	TEAN	0.000	0	0	0	0.00
		Totals	17.988	5857	50111		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
4	4.33		0.000	2526	44419	2	
5	4.62		0.000	1146	12383	2	
10	8.05		0.000	902	36959	2	
12	9.65		0.000	946	12385	2	
		Totals	0.000	5519	106146		

File: IMM11101.D33 Sample: CLAY 84A



Sample Name: SAND 26C

Chromotagram of sandy soil spiked with 26 mg/kg LGP.

Data Reprocessed On 11/11/1993 02:42:31

Sample Name: SAND 26C Date: 11/11/1993 02:05:31
Data File : C:\DX\DATA\IMM11101.D14
Method : C:\DX\METHOD\IMM1110.MET
ACI Address: 1 System: 1 Inject#: 14 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	2.5	3000	5Hz	0.00	10.00	10000

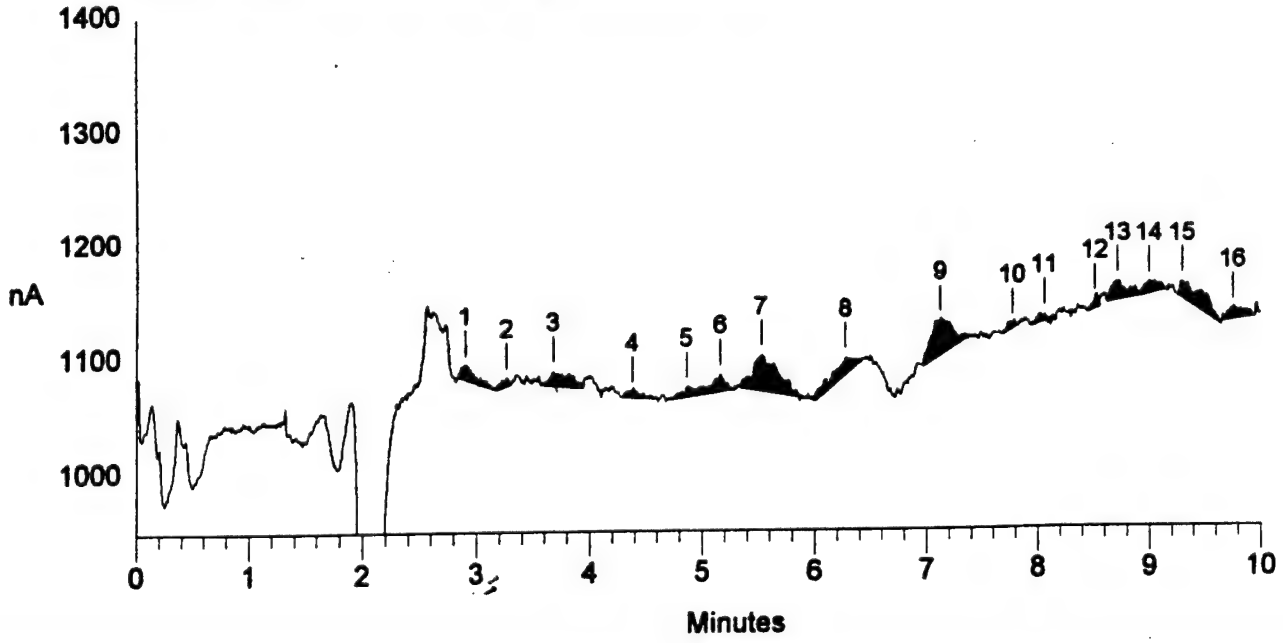
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
3	3.68	HAN	3.989	1221	13624	1	0.00
9	7.13	TEAN	8.740	2935	39026	1	0.00
		Totals	12.730	4156	52649		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
7	5.53		0.000	2986	46398	1	
8	6.28		0.000	1351	18320	1	
13	8.72		0.000	1555	14292	2	
15	9.30		0.000	1381	24057	1	
		Totals	0.000	7273	103068		

File: IMM11101.D14 Sample: SAND 26C



Sample Name: ORGANIC 260C and CLAY 420B

Soil chromatograms indicating interferences caused by soil type. HAN to TEAN ratio of 3.2 was constant in organic and clay soils; however, the clay soil was spiked with more LGP to compensate for dilution effects during extraction. Chromatogram ORGANIC 260C indicates HAN instability in organic soil. HAN was added to a final concentration of 158.6 mg/L, only 6.5 was recovered following extraction of soil immediately after LGP addition. Chromatogram CLAY 420B indicates relatively efficient recovery of the 256.2 and 79.8 mg/kg of HAN and TEAN added. Chromatograms correspond to data shown in Table 20.

Data Reprocessed On 11/11/1993 23:41:00

Sample Name: CLAY 420B Date: 11/11/1993 05:42:05
Data File : C:\DX\DATA\IMM11101.D34
Method : C:\DX\METHOD\imm1110.met
ACI Address: 1 System: 1 Inject#: 34 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	8	3000	5Hz	0.00	10.00	10000

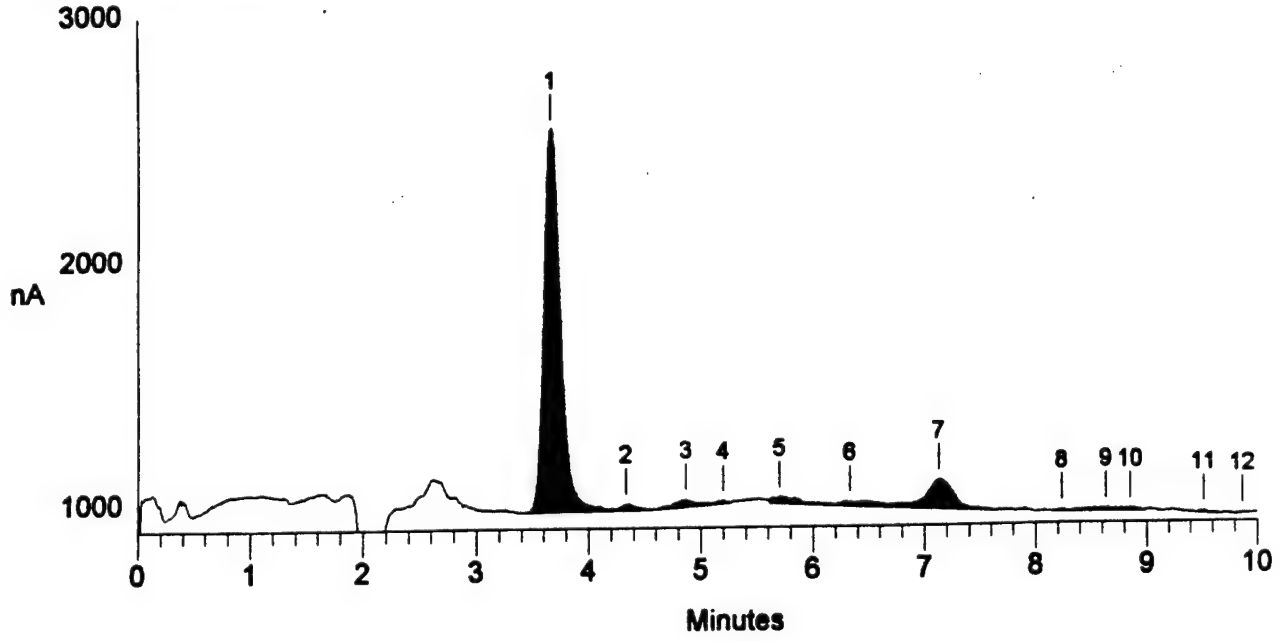
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	3.67	HAN	228.526	156003	1521224	2	0.00
7	7.13	TEAN	59.514	11415	178843	2	0.00
		Totals	288.040	167418	1700067		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
2	4.33		0.000	2156	16701	2	
3	4.87		0.000	2674	34873	2	
5	5.70		0.000	2177	38047	2	
6	6.33		0.000	1474	44240	2	
9	8.63		0.000	1075	16277	2	
10	8.85		0.000	848	11465	2	
		Totals	0.000	10404	161604		

File: IMM11101.D34 Sample: CLAY 420B



Data Reprocessed On 11/12/1993 00:11:43

Sample Name: ORGANIC 260C Date: 11/11/1993 04:59:36
Data File : C:\DX\DATA\IMM11101.D30
Method : C:\DX\METHOD\imm1110.met
ACI Address: 1 System: 1 Inject#: 30 Vial: Detector:PAD
Analyst : J.Rightmyer Column: Waters IC-Pak Cation M/D

Calibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
External	1	2.5	3000	5Hz	0.00	10.00	10000

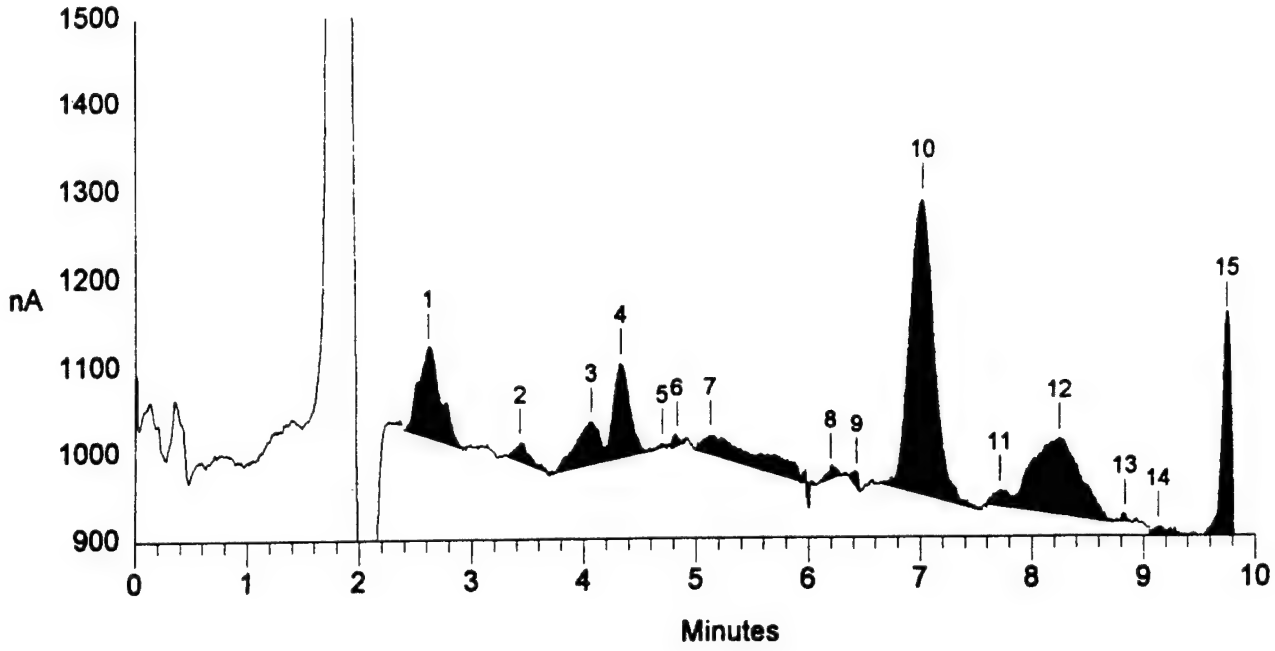
********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
3	4.07	HAN	6.479	4766	69293	2	0.00
10	7.02	TEAN	41.405	33887	502310	1	0.00
		Totals	47.884	38653	571603		

********** Peak Report: Unknown Peaks **********

Pk. Num	Ret Time	Component Name	Concentration ppm	Height	Area	Bl. Code	%Delta
1	2.62		0.000	10233	140555	1	
2	3.43		0.000	1984	18414	1	
4	4.33		0.000	10677	106150	2	
7	5.13		0.000	2138	90626	1	
11	7.72		0.000	1629	15960	2	
12	8.25		0.000	8734	253937	2	
14	9.13		0.000	4787	284039	2	
15	9.75		0.000	50842	541436	2	
		Totals	0.000	91023	1451116		

File: IMM11101.D30 Sample: ORGANIC 260C



Appendix B

Standard Operating Procedures:

Mineral Salts Media and Agar Plates

INTERNATIONAL
TECHNOLOGY
CORPORATION

BIOTECHNOLOGY
APPLICATIONS CENTER

Mineral Salts Media

Standard Operating Procedure

NUMBER: BAC023

Revision 2
January 1994

STANDARD OPERATING PROCEDURE

MINERAL SALTS MEDIA

1.0 Principle

Mineral Salts Media is used for the microbiological testing performed by IT Corporation's Biotechnology Applications Center (BAC) Laboratory in Knoxville, Tennessee. It is the base-liquid used in the medium from which nutrient agar and carbon-free agar are prepared. It is also used to make dilution tubes.

2.0 Equipment

Equipment	Required Mineral Solutions
Erlenmeyer Flask (Size dependent on task) Magnetic stir bars and plate Pipets $(NH_4)SO_4$ K_2HPO_4 1N NaoH pH Electrode Deionized (DI) Water	25% $MgCl_2 \cdot 6H_2O$ 12% $NaH_2PO_4 \cdot H_2O$ .8% $ZnSO_4 \cdot 7H_2O$ 10% $CaCl_2 \cdot 2H_2O$ .02%$CoCl_2 \cdot 6H_2O$ .0001% $CuSO_4 \cdot 5H_2O$ .02% $MnSO_4 \cdot H_2O$ 2.5% $FeSO_4 \cdot 7HO$

3.0 Procedure

1. Into the Erlenmeyer flask of DI water add 2.5 grams $(NH_4)SO_4$ liter (g/L) and 0.36 KH_2PO_4 g/L.

2. Insert stir bar and begin stirring.

3. Using 1 milliliter (mL) pipets, add 1.0 mL/L each of 25% $MgCl_2 \cdot 6H_2O$ and 12% $NaH_2PO_4 \cdot H_2O$.

4. Add 0.2 mL/L of each of the remaining mineral solutions.

5. Calibrate pH electrode in accordance with pH SOP (BAC014).

6. Measure pH of media (it should be approximately 4.2).

7. With a 10 mL pipet, adjust the pH to a range of 6.5 to 7.0 by adding 1 normal (N) NaOH.

4.0 Interferences

None.

5.0 Calculations

None.

6.0 Quality Control Requirements

None.

INTERNATIONAL TECHNOLOGY CORPORATION

BIOTECHNOLOGY APPLICATIONS CENTER

Agar Plates

Standard Operating Procedure

NUMBER: BAC024

Revision 2
January 1994

STANDARD OPERATING PROCEDURE

AGAR PLATES

1.0 Principle

Agar plates are used to enumerate bacteria present in soil and water samples. Total heterotrophic populations are grown up on dilute nutrient agar plates. Contaminant-degrader colonies are cultured on carbon-free mineral salts agar plates supplemented with a specific carbon source.

2.0 Equipment

- Sterile 100 x 10 millimeter (mm) Petri Dishes
- Automatic Plate Pourer
- Erlenmeyer Flasks
- Aluminum Foil
- Mineral Salts Media (BAC023)
- Agar Purified
- Agar granulated
- Nutrient Broth
- Autoclave
- Stir Bars/Stirrer.

3.0 Procedure

1. Into appropriately sized Erlenmeyer flasks, add stir bars, prepare 3 liters (L) of mineral salts media as per the Mineral Salts Media Standard Operating Procedure (BAC023).

2. Label one of the flasks green for the dilute nutrient agar and the other flask red for the carbon free mineral salts agar.

3. Into the green flask, add 45 grams (g) granulated Agar and 6.9 g Nutrient Broth.

4. Into the red flask, add 45 g purified Agar Noble. Stir for 10 minutes.

5. Using aluminum foil, cover the top of the flask. Cover two additional flasks and the plate purer spout and tubing with aluminum foil.

6. Using an autoclavable tub, place all of the flasks and the attachment into the autoclave and sterilize on the liquid cycle for 45 minutes at 121°C.

7. While the autoclave is cycling, load the plate pourer with the 100 x 15mm petri dishes.

8. After autoclaving attach tubing to persistoltic pump.

9. Stir the contents of the red flask for 1 minute, then lift a small corner of the foil on top of the flask, unwrap the peristaltic tubing and run the open end into the hot agar. Be careful not to touch the newly unwrapped portions of the tubing.

10. Purge the agar through the tube into the empty flasks by depressing the manual button on the plate pourer. This is done to eliminate air bubbles in the tube.

11. Attach the pourer spout onto pourer and begin the plate pouring process. Fill each plate with 24 milliliters (mL) of agar.

12. Repeat Steps 9-11 for the **green** flask.

13. Allow the fresh, sterile plates to cool. Then repackage them into the wrappers the empty plates came in and place them in a refrigerator at 4°C.

4.0 Calculations

None.

5.0 Interferences

None.

6.0 Quality Control

Incubate two of each sets of plates to test for sterility. Assign each batch of plates a lot number and record the lot number on the plate package and in the media logbook. Record the results in the logbook.

Appendix C

Biokinetic Constant Calculations

Biokinetic Constants

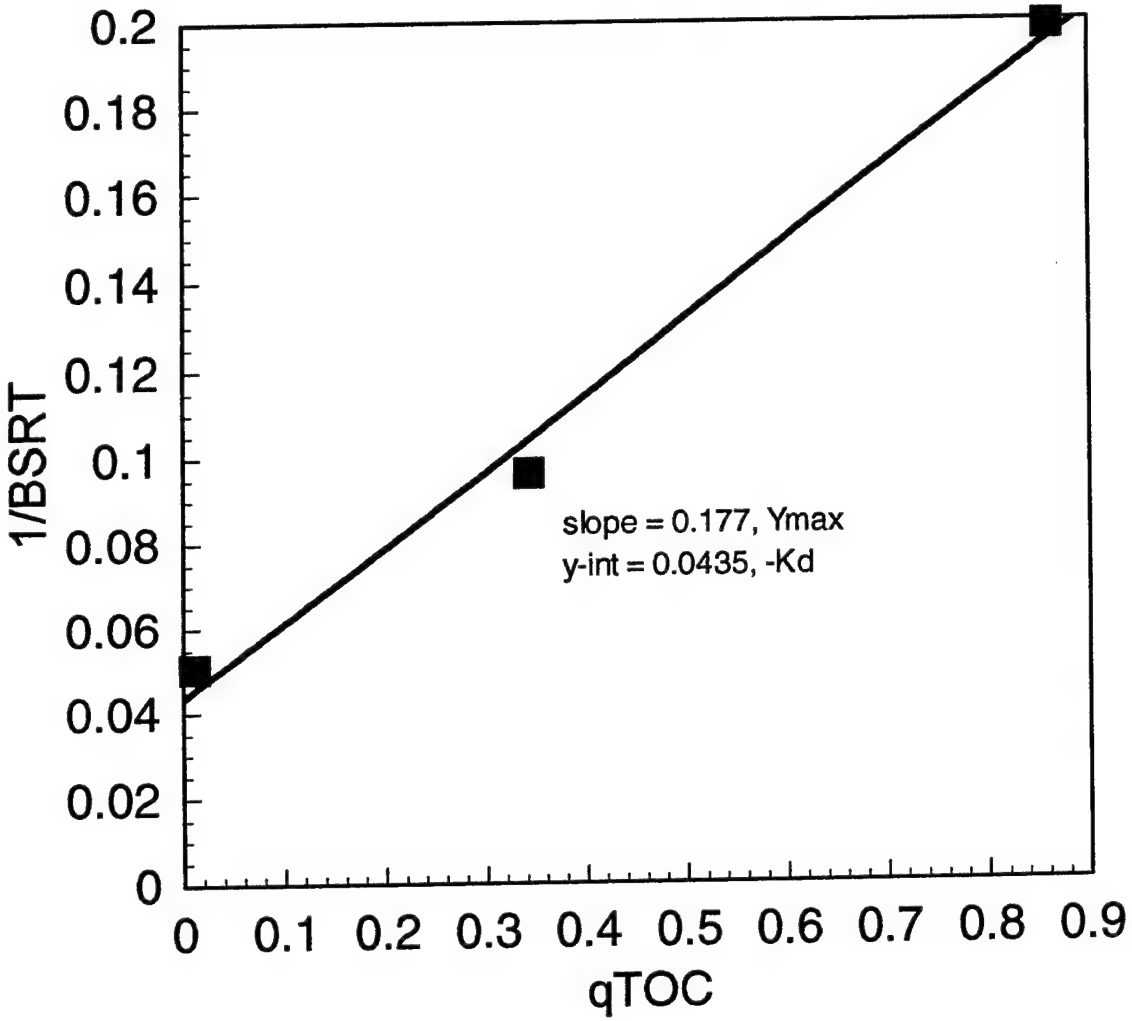
1/BSRT (1/day)	qTOC (mgTOC/ day/mgTSS)	1/BSRT (average)	qTOC (average)	Kd (1/day)	Ymax (mgMLVSS/ mgTOC)	BSRT (days)	Yob (mgMLVSS/ mgTOC)
BSRT = 20 days				*-0.044*	0.1766	20	0.022958
0.0475	0.0048	0.050233	0.010633			10	0.099779
0.0482	0.0122					5	0.1381895
0.055	0.0149						
BSRT=10 days							
0.0927	0.0031	0.096025	0.034175				
0.106	0.0196						
0.088	0.0274						
0.0974	0.0866						
BSRT=5 days							
0.198	0.0038	0.198286	0.085943				
0.215	0.006						
0.193	0.041						
0.194	0.0648						
0.174	0.085						
0.214	0.213						
0.2	0.188						

(1) Kd, microorganism decay rate, 1/day, -Kd = y-intercept of a line generated by plotting 1/BSRT against qTOC

(2) Ymax, maximum sludge yield, mg MLVSS/mg TOC, the slope of a line generated by plotting 1/BSRT against qTOC

(3) Yobs, observed sludge yield, mg MLVSS/mg TOC, Yobs = Ymax(1+BSRT*Kd)

Biokinetic Constants



ESTIMATE OF SPECIFIC SUBSTRATE UTILIZATION RATE CONSTANTS K

USTHAMA

Project : 322240.008

usr1/KT/10/01/94

BSRT	EFF-TOC mg/L	q TOC 1/day	K-TOC L/mg-day	EFF-HAN mg/L	q HAN 1/day	K-HAN L/mg-day	EFF-TEAN mg/L	q TEAN 1/day	K-TEAN L/mg-day
20 DAYS									
FIRST	43.16	0.0048		33.57	0.0871		97.67	-0.0056	
SECOND	38.78	0.0122		38.35	0.1401		101.60	0.0035	
THIRD	57.49	0.0149		157.37	0.5733		113.51	0.0394	
AVERAGE	46.48	0.01060		76.43	0.26681		104.26	0.01246	
SLOPE K			0.00030			0.00380			0.0029
10 DAYS									
FIRST	41.27	0.0031		104.00	0.0342		99.36	-0.0192	
SECOND	34.09	0.0196		67.90	0.1282		92.00	0.0087	
THIRD	36.55	0.0273		90.74	0.2035		100.26	0.0061	
FOURTH	36.66	0.0866		139.90	0.5358		105.40	0.0235	
AVERAGE	37.14	0.03416		100.64	0.22539		99.26	0.00480	
SLOPE K			0.0033			0.0058			0.0033
5 DAYS									
FIRST	37.73	0.0039		105.00	0.0224		120.00	-0.0965	
SECOND	47.90	0.0060		105.00	0.1661		88.00	0.0159	
THIRD	34.14	0.0407		87.40	0.2454		92.80	0.0208	
FOURTH	30.70	0.0651		80.20	0.3530		94.80	0.0174	
FIFTH	30.70	0.0849		76.00	0.4995		96.00	0.0030	
SIXTH	41.80	0.2133		172.00	1.2286		107.00	0.0811	
SEVENTH	41.80	0.1876		172.00	1.0808		107.00	0.0714	
AVERAGE	37.82	0.08592		113.94	0.51372		100.80	0.01617	
SLOPE K			0.0123			0.0085			0.0047

(1) EFF-TOC, -HAN, -TEAN, direct measurement of total organic carbon, HAN, or TEAN in the reactor effluent, mg/L.

(2) qTOC, HAN, TEAN, substrate utilization rate, 1/day.

[(INF TOC - EFF TOC)(Q/Feed Time)/1000 mL/L)/12 hr)]/[(RX VOL/1000 mL/L)TSS RX]

INF TOC = influent TOC, mg/L

EFF-TOC = effluent TOC, mg/L

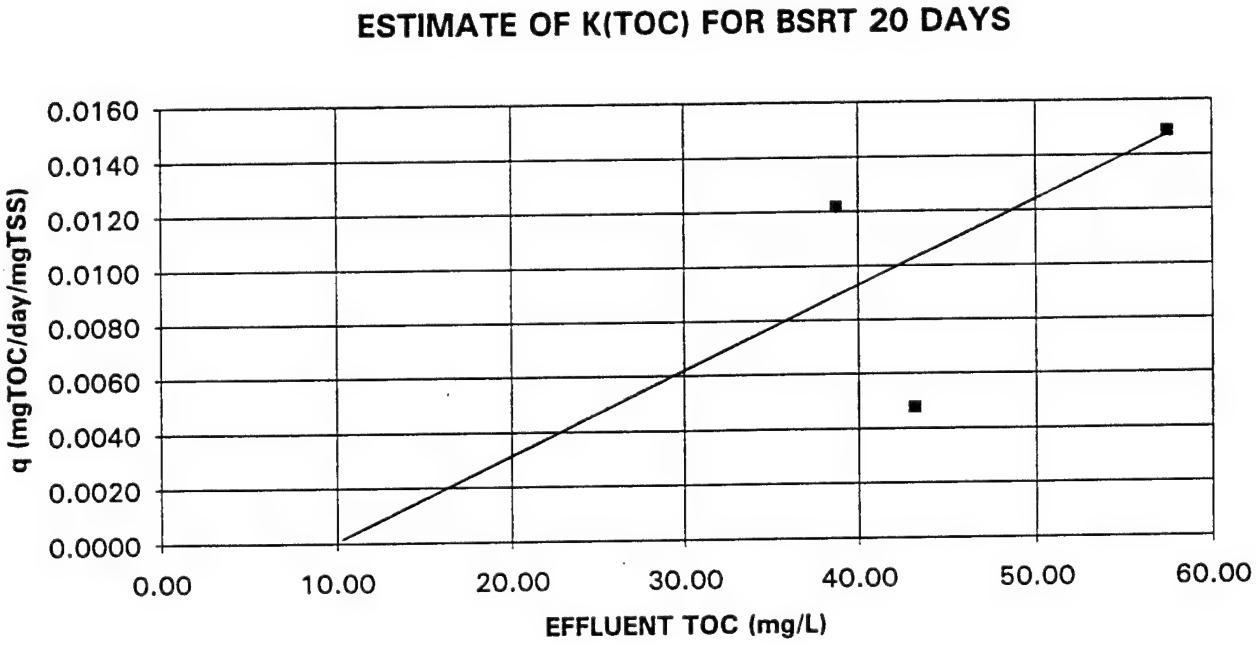
Q = flow rate of feed, mL/min

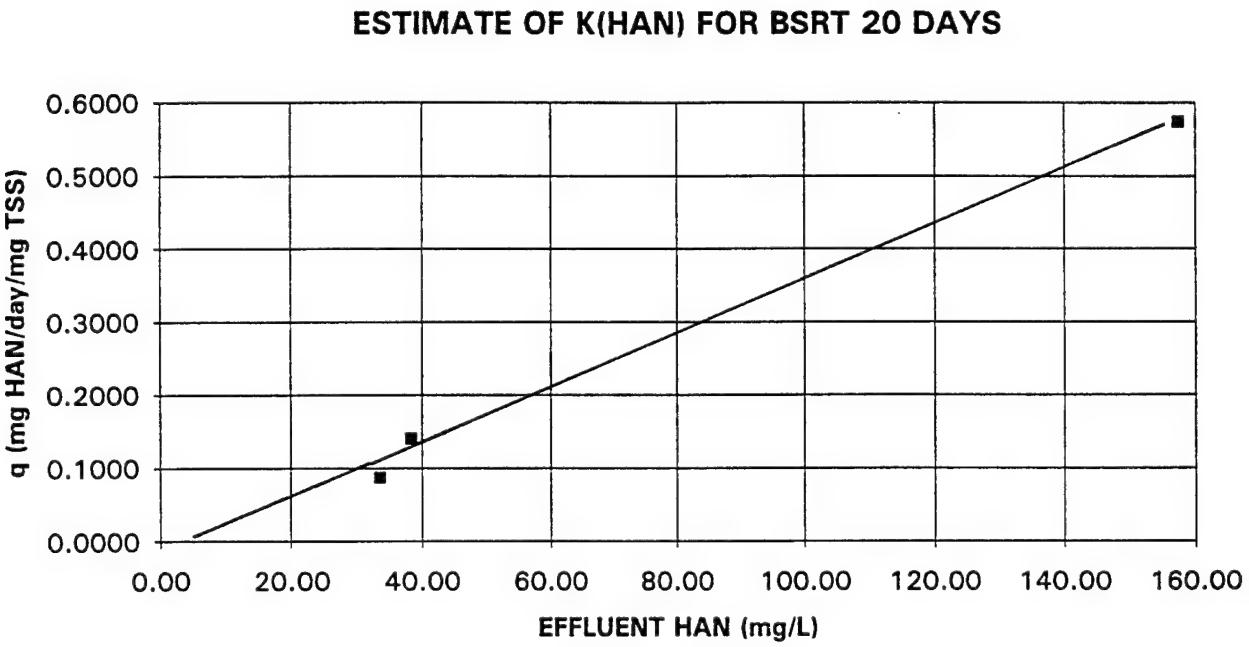
Feed Time = period during batch cycle when feed was added, min

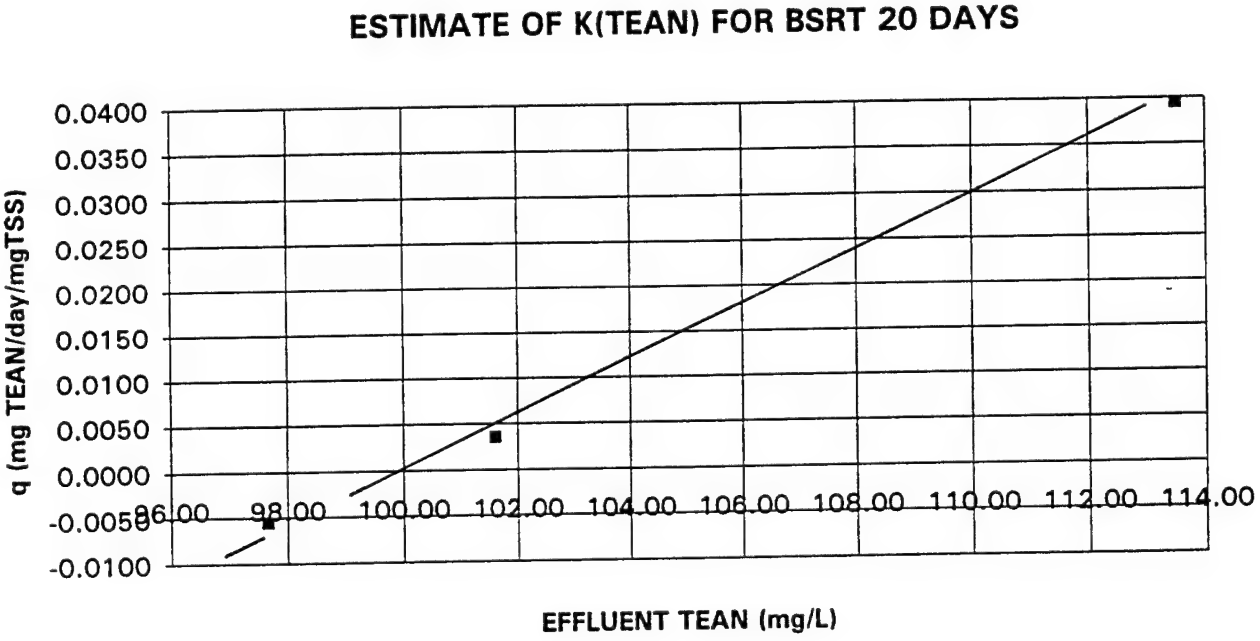
RX VOL = reactor volume, mL

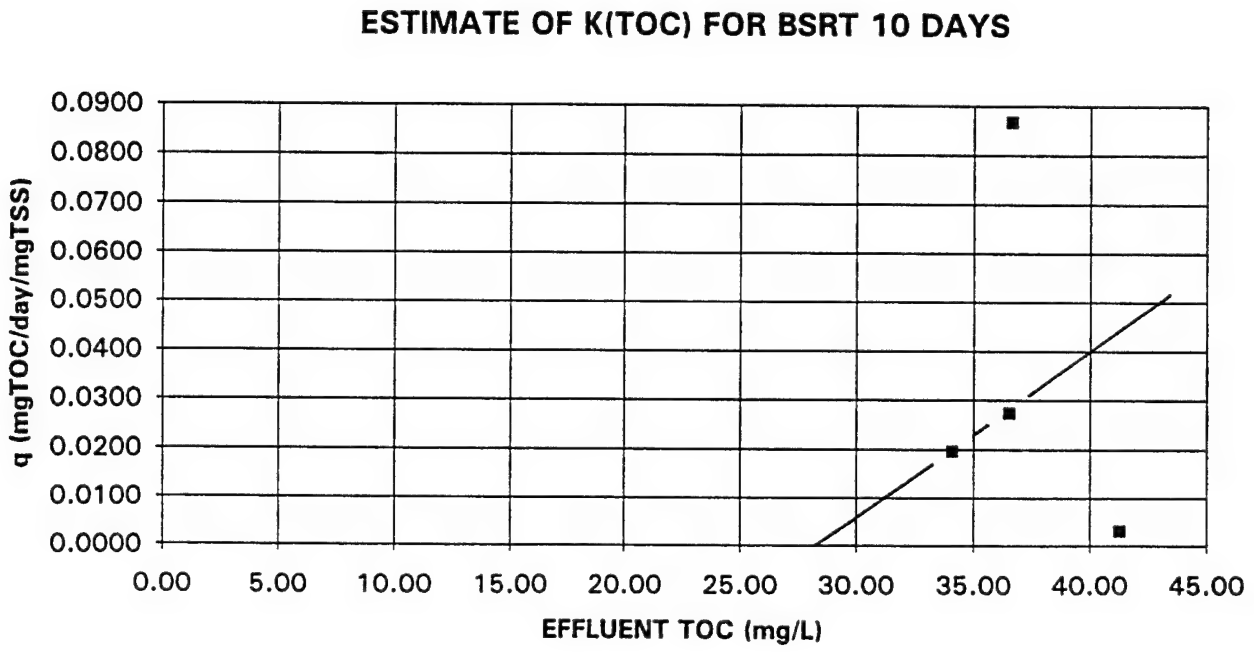
TSS RX = total suspended solids in the reactor, mg/L

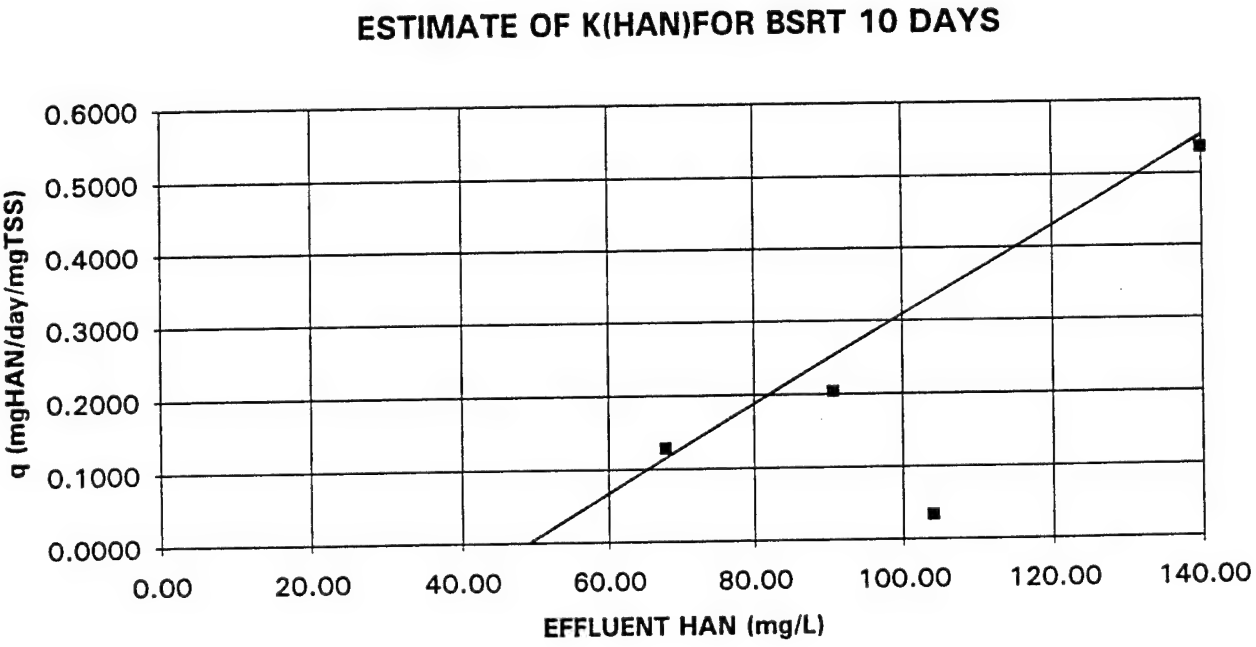
(3) K, specific substrate utilization rate, slope of the line generated by plotting EFF-TOC, -HAN, or -TEAN against qTOC, HAN, or TEAN, L/mg day

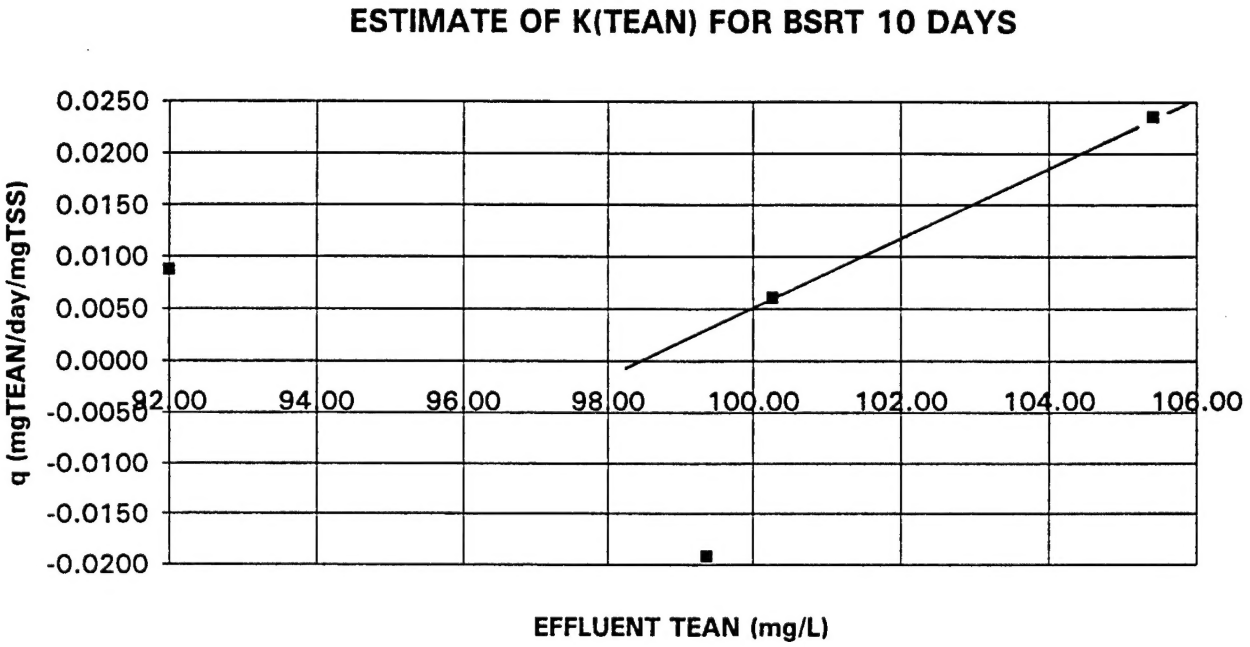

ESTIMATE OF K(TOC) FOR BSRT 20 DAYS
q (mgTOC/day/mgTSS)
0.0160
0.0140
0.0120
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
0.00
10.00
20.00
30.00
40.00
50.00
60.00
EFFLUENT TOC (mg/L)

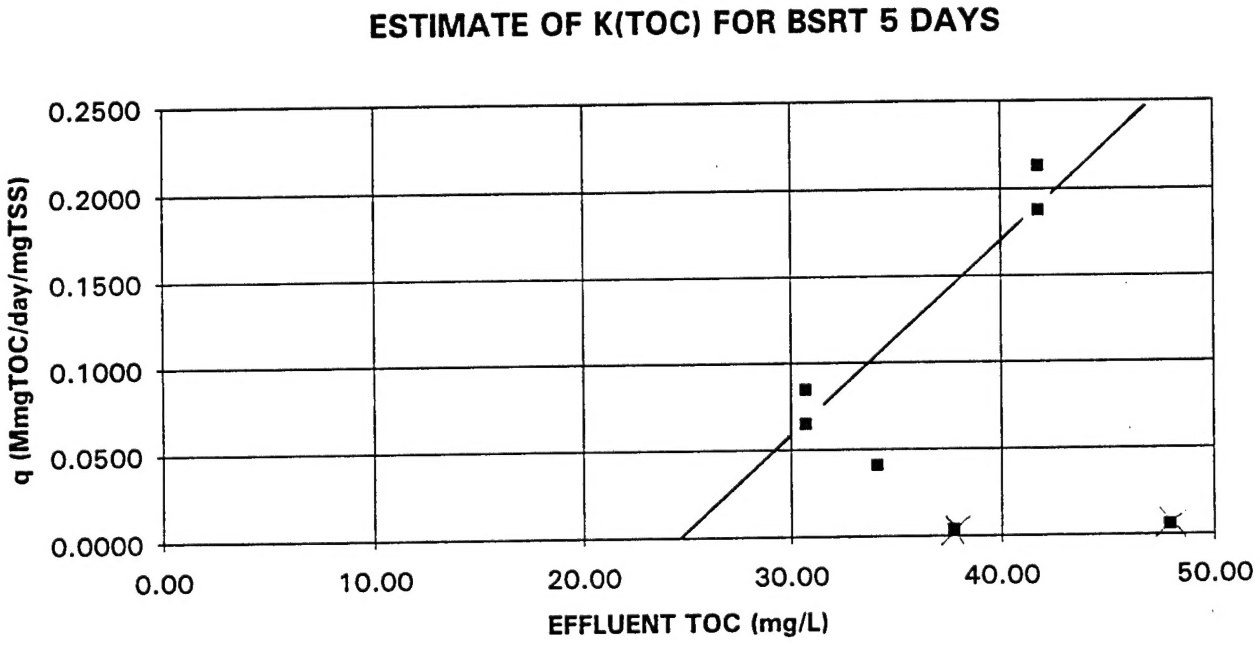

ESTIMATE OF K(HAN) FOR BSRT 20 DAYS
q (mg HAN/day/mg TSS)
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
160.00
EFFLUENT HAN (mg/L)

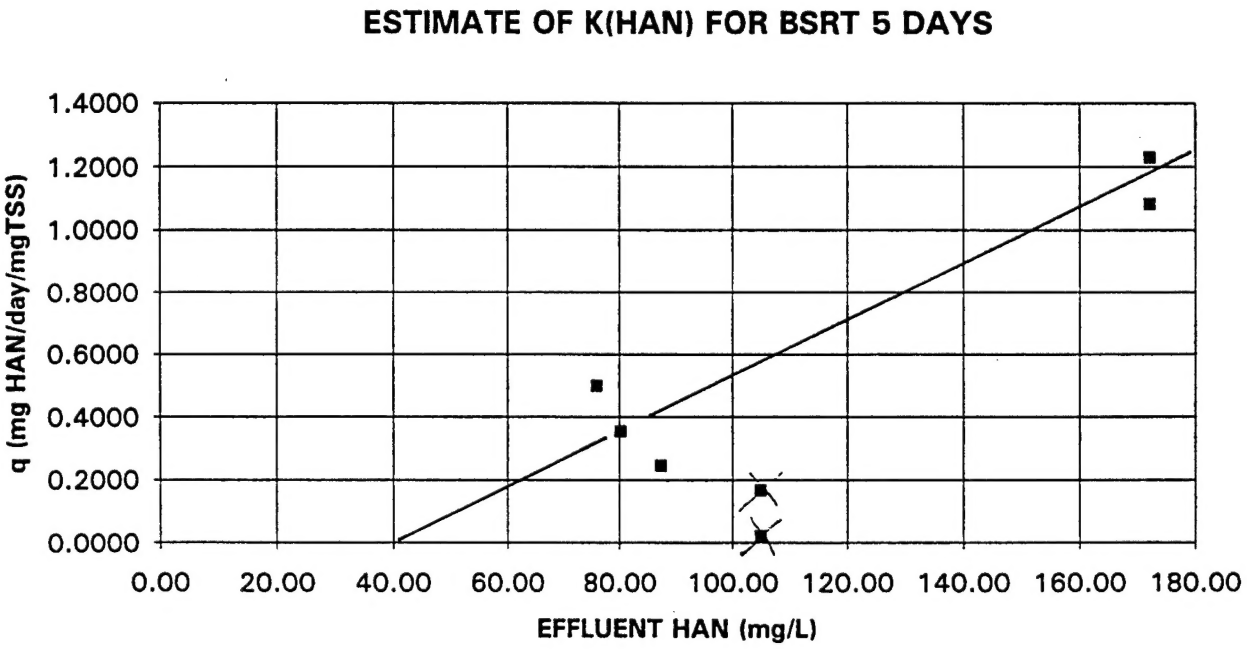

ESTIMATE OF K(TEAN) FOR BSRT 20 DAYS
0.0400
0.0350
0.0300
0.0250
0.0200
0.0150
0.0100
0.0050
0.0000
-0.0050
-0.0100
96.00
98.00
100.00
102.00
104.00
106.00
108.00
110.00
112.00
114.00
q (mg TEAN/day/mgTSS)
EFFLUENT TEAN (mg/L)

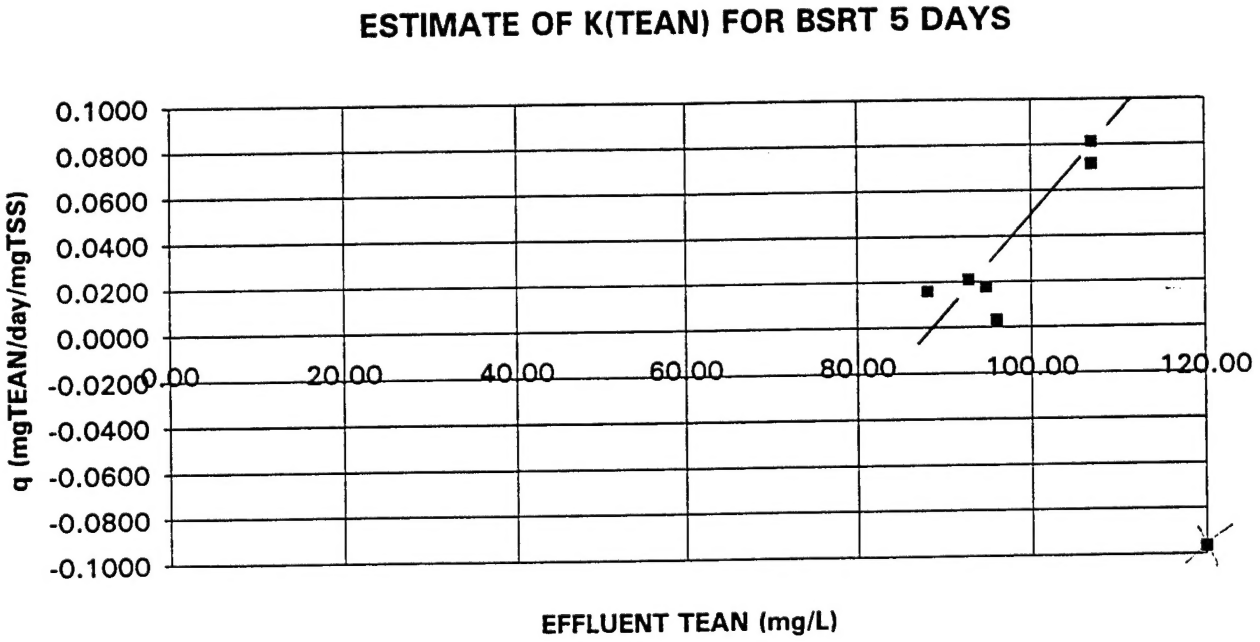

ESTIMATE OF K(TOC) FOR BSRT 10 DAYS
q (mgTOC/day/mgTSS)
0.0900
0.0800
0.0700
0.0600
0.0500
0.0400
0.0300
0.0200
0.0100
0.0000
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
EFFLUENT TOC (mg/L)


ESTIMATE OF K(HAN)FOR BSRT 10 DAYS
q (mgHAN/day/mgTSS)
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
EFFLUENT HAN (mg/L)


ESTIMATE OF K(TEAN) FOR BSRT 10 DAYS
0.0250
0.0200
0.0150
0.0100
0.0050
0.0000
-0.0050
-0.0100
-0.0150
-0.0200
q (mgTEAN/day/mgTSS)
92.00
94.00
96.00
98.00
100.00
102.00
104.00
106.00
EFFLUENT TEAN (mg/L)


ESTIMATE OF K(TOC) FOR BSRT 5 DAYS
0.2500
0.2000
0.1500
0.1000
0.0500
0.0000
q (MmgTOC/day/mgTSS)
0.00
10.00
20.00
30.00
40.00
50.00
EFFLUENT TOC (mg/L)


ESTIMATE OF K(HAN) FOR BSRT 5 DAYS
q (mg HAN/day/mgTSS)
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
160.00
180.00
EFFLUENT HAN (mg/L)


ESTIMATE OF K(TEAN) FOR BSRT 5 DAYS
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
-0.0200
-0.0400
-0.0600
-0.0800
-0.1000
q (mgTEAN/day/mgTSS)
0.00
20.00
40.00
60.00
80.00
100.00
120.00
EFFLUENT TEAN (mg/L)